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A COMPARATIVE STUDY OF BREAD LEAVENED WITH YEAST AND WITH HYDROGEN PEROXIDE [1]

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According to Accum (1821) the use of added yeast in bread making was first (secretly) adopted by French bakers about 250 years ago. The College of Physicians of Paris at first opposed the use of yeast in this way, on the grounds that it was not wholesome. Yeast has nevertheless gradually become the accepted leavening agent for bread throughout most of Europe and the Western Hemisphere. While there are various kinds of leavened bread, none has become so well established and in such general use as that made with yeast. Among the leavened breads that are baked without the use of yeast may be mentioned the following:

(*a*) Salt-rising bread, whose leavening agent is a gas produced by bacteria.

(*b*) So-called aerated bread, the first patent for which, according to Hart (1914), was granted in 1832 to Wright. In 1856, Dauglish introduced the process bearing his name. In this process, which has had considerable success in England and in certain European countries, the water used in doughing is charged with CO_2 under pressure, and the dough mixed in a closed vessel under a pressure of 40 lbs. The heat of the oven suffices to produce a well-leavened loaf. Such a bread quite naturally lacks the flavor of yeast-risen bread and never has become a serious competitor of bread made from fermented dough.

(*c*) Baking powder breads, as the name implies, are made by the use of baking powders as the source of gas, but the grain, texture, volume, and aroma of such breads are inferior to that produced from yeast.

Recently experiments were conducted by the authors to test the

[1] Food Research Division Contribution No. 278.

TABLE I

ANALYSES OF FLOUR AND BREADS MADE THEREFROM

	On fresh basis							On water-free basis						
	Flour 325 g. %	Yeast bread. Wt. of loaf 478 g.			Hydrogen peroxide bread. Wt. of loaf 464 g.			Flour %	Yeast bread			Hydrogen peroxide bread		
		Crust 36.97 %	Crumb 63.03 %	Loaf 100 %	Crust 38.67 %	Crumb 61.33 %	Loaf 100 %		Crust 45.38 %	Crumb 54.62 %	Loaf 100 %	Crust 45.72 %	Crumb 54.28 %	Loaf 100 %
Moisture, %	12.77	20.60	43.80	35.22	19.40	39.90	31.97	—	—	—	—	—	—	—
Ash, %	0.43	1.56	1.21	1.34	1.66	1.21	1.38	0.49	1.96	2.15	2.06	2.06	2.01	2.03
Fat (acid hydrolysis), %	1.95	3.65	2.53	2.94	3.79	2.70	3.12	2.25	4.60	4.50	4.55	4.70	4.49	4.59
Sugars (total), %	1.43	2.11	1.63	1.81	6.54	5.05	5.63	1.64	2.66	2.90	2.79	8.11	8.40	8.27
Sugars (reducing), %	—	2.02	1.62	1.77	2.84	2.12	2.40	—	2.54	2.88	2.73	3.52	3.53	3.53
Nitrogen (total), %	2.30	1.97	1.36	1.58	1.94	1.38	1.60	2.64	2.48	2.42	2.44	2.41	2.30	2.35
Nitrogen (70% alcohol sol.), %	1.14	0.22	0.14	0.17	0.48	0.50	0.49	1.31	0.28	0.25	0.26	0.60	0.83	0.72
Nitrogen (5% pot. sulp. sol.), %	0.28	0.66	0.37	0.48	1.45	1.10	1.24	0.32	0.83	0.66	0.74	1.80	1.83	1.82
Nitrogen (1% sod. chlor. sol.), %	0.40	0.11	0.07	0.08	0.26	0.21	0.23	0.46	0.14	0.13	0.13	0.32	0.35	0.34
Nitrogen (water soluble), %	0.42	0.10	0.07	0.08	0.31	0.24	0.27	0.48	0.13	0.13	0.13	0.38	0.40	0.39
Starch (water soluble), %	1.86	2.94	1.27	1.89	1.82	1.36	1.54	2.13	3.70	2.26	2.91	2.26	2.26	2.26
Solids (water soluble), %	8.38	10.03	6.40	7.74	13.90	10.66	11.91	9.61	12.63	11.39	11.95	17.24	17.74	17.51

possibility of using dry ice as the source of gas in the leavening of bread dough, but thus far without success.

More recently still, experiments in the making of bread have been conducted by the use of what is known in the trade as "100 vol. (30%) hydrogen peroxide" *as the sole* leavening agent.[2]

Experimental

In making a pound loaf of bread by this method, about 25 cc. of hydrogen peroxide is added to the dough toward the end of the mixing process. The addition of sugar, milk, shortening, malt, etc., is, of course, not essential, except possibly for flavor. After the peroxide has been thoroughly incorporated into the dough, the latter is immediately placed in the baking pan and allowed to rise overnight at ordinary room temperature. It is essential to prevent the dough from forming a crust. At the time the dough is transferred to the pan, a small piece is set aside, and from time to time is tested for peroxide, using a spot-plate and a few drops of potassium iodide solution. This piece also should be prevented from crusting. From our experience it is necessary to let the reaction proceed about 12 to 16 hours before the hydrogen peroxide has been fully decomposed. When no further blue color is noted with the potassium iodide solution, the panned dough is placed in the oven.[3]

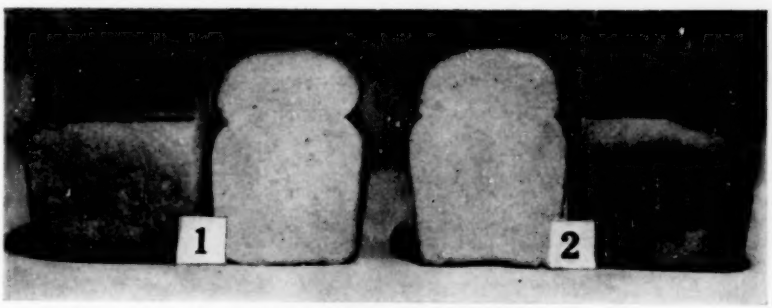

Fig. 1. (1) White bread leavened with yeast.
(2) White bread leavened with hydrogen peroxide.

The bread thus made has a volume and general appearance equal to that of yeast bread and possesses a grain and texture fully as good as if not superior to that of ordinary bread. Loaves which were leavened with H_2O_2 and yeast are shown as Figures 1 and 2.

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[2] This investigation was instigated as the result of some experimental work that was carried on in cake making in collaboration with the R. & H. Chemicals Department of the E. I. Dupont de Nemours & Co.

[3] The basic bread formula used was 325 g. flour, 10 g. sugar, 8 g. yeast, 7 g. shortening, 5 g. salt, 193 cc. H_2O. The hydrogen peroxide bread was made by using 25 cc. of peroxide to replace the 8 g. of yeast. When using peroxide, of course, a correspondingly smaller amount of water is needed for doughing purposes.

In one respect, however, hydrogen peroxide bread is much inferior to yeast-risen bread, *viz.*, in flavor and aroma,[4] qualities which are the result largely of yeast fermentation. Both crust and crumb are extremely tender.

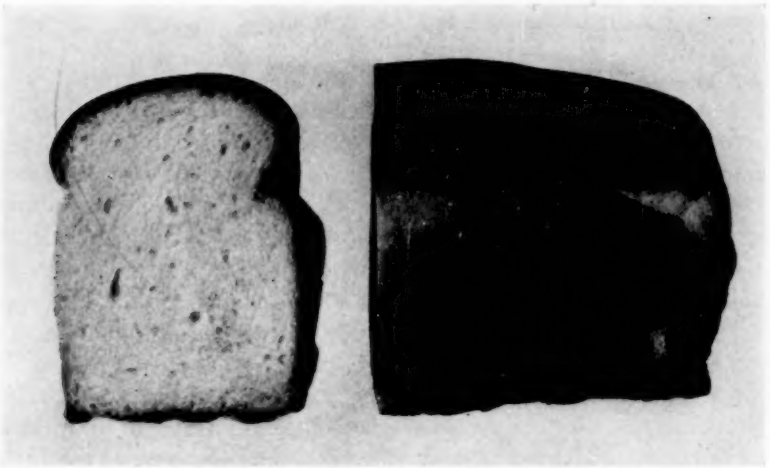

Fig. 2. General appearance of exterior and interior of H_2O_2 leavened bread.

In making hydrogen peroxide bread, the essential equipment is a dough mixer, storage space for dough while proofing in pans, ovens, and cooling racks. There is no need for complicated and expensive conditioning systems, nor for special temperature controls during fermentation and proofing. The composition of the breads is given in Table I, page 120.

Both the yeast and peroxide breads were weighed one hour after baking, and separated as completely as practicable into crust and crumb. Each was analyzed for the constituents recorded in Table I.

Discussion

The weight of the original yeast and hydrogen peroxide doughs was 541 and 536 g. respectively. In the process of leavening by yeast action, the loss in the weight of dough amounted to 1.0%; as the result of peroxide decomposition, the loss was 1.3%. During the baking process and cooling for one hour a further loss of 10.6% and 12.1% respectively was noted. The weight of the crust and crumb in each type of bread was as follows:

[4] A very good, attractive, and tasty bread can be made by using up to one-third as much cheese as flour.

TABLE II

WEIGHT AND PERCENTAGE OF CRUST AND CRUMB OF BREAD

Portion of loaf	Fresh basis (One hour old)		Water-free basis	
	Yeast	Hydrogen peroxide	Yeast	Hydrogen peroxide
Crust, g.	177.0	179.0	140.5	144.3
Crumb, g	301.0	285.0	169.2	171.3
Loaf, g.	478.0	464.0	309.7	315.6
Crust, %	37.0	38.7	45.4	45.7
Crumb, %	63.0	61.3	54.6	54.3

TABLE III

MOISTURE IN BREAD (ONE HOUR OLD)

Portion of loaf	Yeast bread		Peroxide bread	
	Grams	%	*Grams*	%
Crust,	36.4	20.6	34.8	19.4
Crumb,	132.0	43.8	113.0	39.9
Whole loaf,	168.4	35.2	147.8	32.0

The peroxide bread contained 38.7% of crust and 61.3% crumb, or slightly more crust and correspondingly less crumb than the yeast bread. The moisture content of yeast bread was appreciably higher than that of peroxide bread (35.2 and 32.0 respectively).

The analytical data show the differences in the composition of breads made (*a*) with yeast and (*b*) with peroxide.

Composition of Flour and Bread

As is to be expected, the amount of minerals, fat, and protein of flour suffers little if any change during the process of bread baking. With sugar, however, it is different. Here the enzymes of flour and yeast play a very active role. The sugar in the dough (both that preexisting and that added) is for the most part converted by the zymase of the yeast into carbon dioxide and alcohol. To what extent the starch of the flour is converted into sugar by the diastase present and how much of this sugar is subsequently converted into carbon dioxide and alcohol varies, of course, with conditions.

Some of our unpublished data indicate that for every 100 g. of flour subjected to yeast-fermentation, as in the making of bread, approximately 4 g. of starch are converted into sugar. Geddes and Larmour (1933) found, during fermentation, an amount of CO_2 which would indicate that even as much as 5 g. of starch are converted into

sugar by enzymes in the dough. That the presence of hydrogen peroxide is no hindrance to the action of enzymes in flour-water mixtures is evidenced by the large amount of sugar found in peroxide bread.

TABLE IV

COMPOSITION OF INGREDIENTS

	Flour—325 g.		Yeast[1]—8 g.		Shortening—7 g.		Sugar—10 g.		Salt—5 g.		Total constituents	
											Yeast bread [2]—(478 g.)	H_2O_2 bread [3]—(464 g.)
	%	*Grams*	%	*Grams*	%	*Grams*	%	*Grams*	%	*Grams*	*Grams*	*Grams*
H_2O	12.77	41.50	73.0	5.84	—	—	—	—	—	—	240.34	234.50
Ash	0.43	1.40	2.4	.19	—	—	—	—	100.	5.0	6.59	6.40
Fat	1.95	6.34	0.3	.02	100.	7.0	—	—	—	—	13.36	13.34
Sugar	1.48	4.66	—	—	—	—	100.	10.0	—	—	14.66	14.66
Protein	13.11	42.60	15.0	1.20	—	—	—	—	—	—	43.80	42.60

[1] Approximate average. Ward Baking Co. (N. Y. 1922), Res. Prod. Dept. Proper Yeast Nutrient Salts.
[2] Includes 193 cc. H_2O used in doughing.
[3] Includes 168 cc. H_2O + 25 cc. H_2O_2.

TABLE V

AMOUNT OF THE VARIOUS CONSTITUENTS IN THE TOTAL INGREDIENTS USED IN MAKING BREAD AND IN BREAD ITSELF

	Yeast bread (478 g.)		H_2O_2 bread (464 g.)	
	Ingredients	Bread	Ingredients	Bread
	Grams	*Grams*	*Grams*	*Grams*
H_2O	240.34	168.76	234.50 [1]	144.80
Ash	6.59	6.45	6.40	6.45
Fat	13.36	14.05	13.34	14.48
Sugar	14.66	8.70	14.66	26.16
Protein	43.80	43.07	42.60	42.32
70% alc. sol. N	3.70 [2]	.81	3.70	2.32
5% K_2SO_4 sol. N	.91 [2]	2.26	.91	5.75
1% NaCl sol. N	1.30 [2]	.38	1.30	1.07
H_2O sol. N	1.36 [2]	.38	1.36	1.21
H_2O sol. starch	6.04 [2]	8.65	6.04	7.15
H_2O sol. extract	27.23 [2]	36.71	27.23	55.45

[1] Includes H_2O_2.
[2] Yeast solubles not included (amount not appreciable).

The changes in the amount of the various protein fractions are significant. The amount of alcohol soluble nitrogen in the 325 g. of flour was 3.70 g. while in the 478 g. of fresh yeast bread and 464 g. of fresh peroxide bread the amount was 0.81 g. and 2.32 g. respectively. Evidently the yeast is an important factor in bringing about these differences.

The amount of potassium sulphate-soluble nitrogen was much less in the flour (0.91 g.) than in the two types of bread, indicating

that this type of protein decomposition products is considerably increased by the proteolytic enzymes of the flour. That the peroxide bread contains more than twice as much of the potassium sulphate-soluble nitrogen as does yeast bread is probably due to the fact that the proteolytic enzymes of the peroxide bread had been active for 15 hours instead of three hours as in the case of the yeast bread.

The water-soluble and salt-soluble nitrogen of flour are very little affected in making peroxide bread but considerably so when yeast bread is made. As a whole the peroxide bread has a much higher percentage and amount of protein decomposition products than has yeast bread.

The bread made with hydrogen peroxide contained, on the dry basis, more than 8% of sugar; that made with yeast only 3%. On the other hand, the amount of soluble starch in yeast bread is somewhat higher than in the peroxide bread. It is evident that the amount of soluble starch found in yeast bread is only a part of the actual amount formed by the action of the enzymes of flour. From the data presented, at least 17.5 g. of starch must have been converted into sugar during the process of fermentation. In the yeast bread, a large part of this sugar was further converted into alcohol and carbon dioxide.

The crust of the yeast-bread is appreciably richer in soluble starch than is the crumb, due to the action of the enzymes of the yeast upon the starch and to the heat of the oven. The enzymes of the flour itself are evidently not such as to yield the same intermediate compounds as are produced by yeast enzymes, for the crust of the peroxide bread is no richer in soluble starch than is the crumb.

In other respects the crust and crumb of the yeast bread have the same composition.

Summary

Breads were made with yeast in the usual way, and with hydrogen peroxide in place of yeast.

These breads were divided into crust and crumb, and each was separately analyzed for ash, fat, reducing sugar, total sugar, water-soluble solids, water-soluble starch, total nitrogen, water-soluble nitrogen, alcohol-soluble nitrogen, 5% potassium sulphate-soluble nitrogen, and 1% sodium chloride-soluble nitrogen.

The peroxide bread made had slightly more crust than the yeast bread, and contained less moisture.

The crust of the yeast bread is appreciably richer in soluble starch than is the crumb. In other respects the composition of the crust and crumb of the yeast bread (on water-free basis) is practically the same. Likewise, the crust and crumb of the peroxide bread have essentially the same composition.

In general appearance of crumb, the two kinds of breads were much alike. The crust of yeast bread is, however, as a rule, more attractive. The bread made with peroxide contained approximately three times as much sugar, alcohol-soluble nitrogen, potassium sulphate soluble nitrogen, sodium chloride-soluble nitrogen, and water-soluble nitrogen as did the yeast bread. It contained but slightly less soluble starch. The percentage of fat, ash, and total nitrogen in both kinds of breads was the same. The peroxide bread has as good a grain and fully as good a volume as the corresponding yeast bread, and a better texture. In taste, the peroxide bread was inferior to the yeast bread.

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STUDIES ON TOXIC WHEAT GROWN ON SOILS CONTAINING SELENIUM [1]

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Introduction

As the result of experiments carried on by Kurt W. Franke of the South Dakota Agricultural Experiment Station, the attention of Henry G. Knight, Chief of the Bureau of Chemistry and Soils, was directed about four years ago to a disease of obscure origin that occurred in farm animals. This disease, known locally, but erroneously, as "alkali disease," was traced by Franke to feedingstuffs grown upon certain localized areas of soils. Grains, including corn, wheat, barley, and emmer, grown on these areas were found to be toxic to small experimental animals, white rats, and produced pathological symptoms unlike those produced by any of the known plant toxins.

In the early part of 1931, cooperative studies were undertaken by four Bureaus of the United States Department of Agriculture and the South Dakota Agricultural Experiment Station. A preliminary survey made in the summer of that year through the central and southwestern

[1] This paper in its essential features was read at the Annual Meeting of the American Association of Cereal Chemists, June, 1935.

[2] The authors wish to acknowledge the assistance of S. Phillips of their Division in the preparation of some of the material used in this investigation.

parts of South Dakota, parts of northern Nebraska, and the eastern edge of Wyoming showed that this disease occurs in localized areas among horses, cattle, swine, and poultry. The results of this joint survey have been recently described in an article published by Franke, Rice, Johnson, and Schoening (1934). The disease manifests itself in cattle, horses, and swine by loss of appetite, weakness, loss of hair, and, in severe cases, abnormal growth of the hoofs, resulting eventually in lameness and sloughing off of the old hoofs. The disease in poultry is manifested by a marked lowering of the hatchability of the eggs. The small proportion of young that do hatch are weak and abnormally developed.

Soon after the cooperative agreement with the South Dakota Agricultural Experiment Station, feeding experiments with white rats were started in the Protein and Nutrition Division of the Bureau of Chemistry and Soils. The object of these experiments was to develop information relative to the nature and character of the factor which produces the toxicity in the grains referred to, and to identify and isolate the specific offending compound.

All the feeding experiments described in this paper were completed in 1931, except those made on the wheat plants grown at Arlington, which were completed in 1932.

The discovery by Robinson (1933) of the presence of selenium in the toxic wheat which we had been using in our biological studies, and the observations referred to later in this paper that selenium in the soil is assimilated by plants with the elaboration of a compound toxic to experimental animals such as rats and guinea pigs, prompted the publication of a note in Science by Nelson, Hurd-Karrer, and Robinson (1933) calling attention to the possible danger attending the use of selenium compounds as insecticides.

Since most of the work herein described was completed, a number of papers have appeared from the United States Department of Agriculture and from the South Dakota Agricultural Experiment Station on different phases of the selenium problem. The status and significance of the selenium problem in its general aspects have been reviewed by Knight (1935). In a recent bulletin, Byers (1935) has given an account of the extensive work done in this Department of the occurrence and distribution of selenium in certain soil areas injuriously affected by its presence. Work done in the Bureau of Plant Industry by Hurd-Karrer (1934, 1935) has dealt with absorption of selenium from the soil by plants, the injury done by it to wheat plants, and inhibition by sulfur in the soil.

Feeding experiments conducted in the Bureau of Home Economics by Munsell and DeVaney showed that inorganic selenium salts produced symptoms in rats similar to those produced by toxic grain.

From the South Dakota Agricultural Experiment Station several papers have been published by Franke and co-workers (1934, 1935) on the chemical and biological phases of the subject. It is of interest to note that, although working independently, there is a close agreement in the general results obtained by them with those obtained in this laboratory with respect to many of the findings relating to the general nature and effects of the toxic substance present in the wheat investigated.

Feeding Experiments [3]

The toxic wheat employed in our feeding experiments was supplied by K. W. Franke. In all the growth studies we used Sherman's diet, which consisted of 66 percent finely ground whole wheat, 33 percent whole milk powder, and 1 percent sodium chloride. The general procedure was to compare the performance of animals fed this ration made from normal constituents with litter mate animals fed the same ration made with wheat suspected of being toxic. Four animals were used in each group, with the exception of the first two experiments, for which the supply of wheat was too limited to feed this number of animals. With these exceptions, the growth curves shown represent the average performance of four animals. The animals were kept in individual cages, and food consumption records were maintained.

The first experiment was designed to show whether the toxicity of the wheat was due to the presence of some foreign substance or to some constituent of the wheat which could not readily be separated mechanically. The toxic wheat was carefully separated from foreign material by hand, and the clean grain was ground and incorporated in the diet. The growth response of individual rats fed control wheat, the toxic wheat as received, and the cleaned wheat, is shown in Figure 1. The toxic principle apparently was not removed with the foreign material.

In order to determine whether the toxic principle was appreciably soluble in water, alcohol, or ether, portions of the ground wheat were extracted in the cold with the above-mentioned solvents. The extracts obtained were dried on a quantity of normal wheat equal to that extracted, and this material was used to make up the standard ration. The extracted residues were dried and made into similar rations. That the solvents did not remove demonstrable quantities of the toxic principle is shown by the results obtained when these materials were fed (see Figure 1). The extracts did not retard the growth of the animals, and the extracted residues were all quite effective in retarding growth.

In order to get information on the distribution of the toxic principle in the different parts of the wheat, quantities of normal and of toxic

[3] A preliminary report of the results of the feeding experiments was presented by E. M. Nelson and D. B. Jones to the American Institute of Nutrition at the Second Annual Meeting held in Detroit, April 10, 1935. (Abstract, J. Nutrition, V, No. 6, and Suppl., June 10th.)

wheat were milled.[4] The toxic grain yielded: Flour, 58 percent; bran, 27 percent; and middlings, 15 percent. The normal wheat similarly

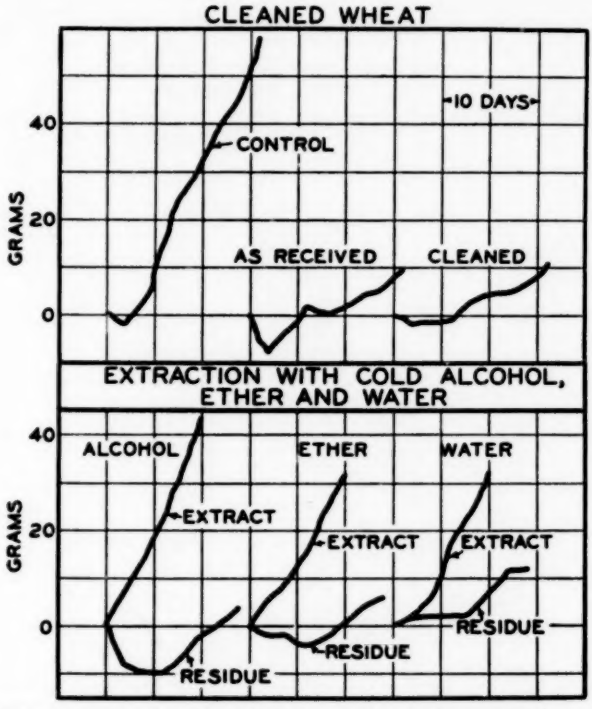

Fig. 1. These curves show that the mechanical removal of foreign material from the toxic wheat as received did not remove the toxic principle, and that it is not appreciably soluble in the solvents indicated.

milled yielded: Flour, 59 percent; bran, 27 percent; and middlings, 14 percent. These products were then used in the following combinations to replace the wheat in the basal diet for five groups of animals:

1. The normal wheat was reconstituted from the flour, bran, and middlings obtained.

2. The same as No. 1, except that the middlings of the control wheat were replaced by the middlings from the toxic wheat.

3. The same as No. 1, except that the bran from the control wheat was replaced by bran from the toxic wheat.

4. The same as No. 1, except that the flour from the normal wheat was replaced by flour from the toxic wheat.

5. The toxic wheat was reconstituted from its milling products.

The growth curves in Figure 2 show that the toxic principle was distributed fairly uniformly in the flour, bran, and middlings. The

[4] The milling products of the normal and toxic wheat used in the experiments described in this paper were prepared for us in the experimental flour mill of the Food Research Division of this Bureau.

retardation of growth was greater where the normal flour was replaced by the toxic flour than when the bran or middlings were replaced by toxic materials, but this is due to the fact that the ration contained a higher percentage of toxic material.

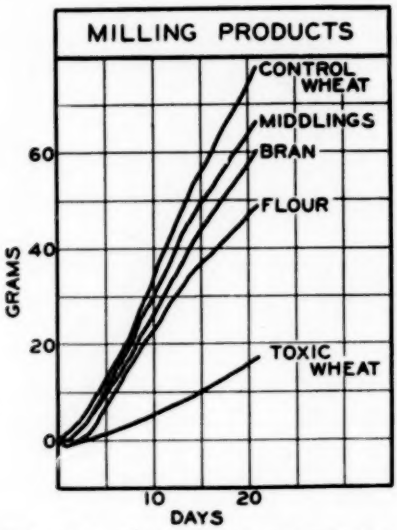

Fig. 2. Distribution of the toxic factor in the milling products of toxic wheat.
These graphs show that the toxic principle is fairly uniformly distributed in the flour, middlings, and bran.

At this point in the investigation it was not known whether the toxicity was to be attributed to an organic or inorganic constituent of the wheat. The low percentage of inorganic material present in the flour made it possible to feed this material at a level equivalent to that contained in a large proportion of the flour without introducing conflicting factors. Accordingly, 500 g. samples of normal and toxic flours were reduced to a gray ash in a muffle at a temperature not exceeding 500° C. The normal and toxic flours yielded respectively 2.3676 and 2.0465 g. ash. The results obtained by feeding the ash preparations incorporated in the basal diet at a level equivalent to that represented by 80 percent of flour show that the ash prepared from the toxic flour as described did not have the growth-retarding property corresponding to that of the equivalent amount of the toxic flour.

The fact that the protein of wheat flour can readily be separated as gluten provided an opportunity to make a further investigation of the flour. Five hundred grams of toxic flour yielded 74 g. of dry starch-free gluten, and an equal quantity of control flour yielded 56 g. of gluten. These preparations were substituted for wheat in the basal diets, and each diet contained 20 percent of gluten, as shown in Figure 3.

This level of gluten from toxic flour prevented an increase in weight of the animals while the growth of the control animals was typical of normal response. These results indicate that the toxic principle of the flour is resident entirely in the protein fraction.

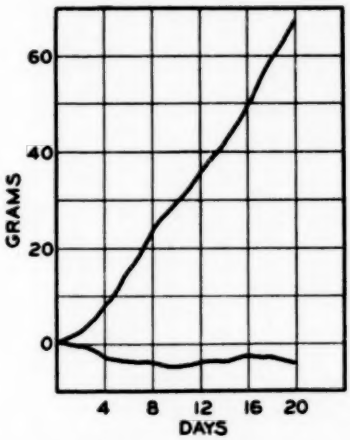

Fig. 3. The toxic principle of the flour is resident in the protein.
The upper curve shows the average rate of growth of rats fed a ration containing 20 percent of gluten from normal wheat. The lower curve similarly shows the rate of growth when the same quantity of gluten from toxic wheat was used.

While the studies described were being conducted, other lines of investigation were in progress in the Department of Agriculture. One of these was an attempt to detect the presence of an abnormal quantity of some unusual inorganic element in toxic grain. All attempts gave negative results until, at the suggestion of Dr. Knight, Robinson (1933) examined such grain for selenium. He found that the wheat which we had been using in our biological studies contained approximately 10 parts per million of selenium and that this element could not be detected in a number of other samples of wheat. A portion of the toxic gluten which we had used in our feeding tests was found to contain 90 parts per million of selenium.

In the studies already referred to in which the ash of flour proved to be nontoxic the selenium was volatilized during the ashing procedure. The selenium can be fixed by suitable addition of calcium acetate before ashing. One and one-half grams of calcium acetate were added to 15 g. samples of toxic and nontoxic gluten, and the material was reduced to a gray ash in an electric muffle. This material was then added to the basal diet in amounts equivalent to 15 percent of gluten and fed to one group of animals, while two comparable groups received, respectively, 15 percent of normal and toxic gluten. It can be seen in Figure 4 that

the ash from toxic gluten has some growth-retarding effect, but the influence on growth is slight compared to the effect produced by the gluten itself. If there was no loss of selenium due to ashing, it appears

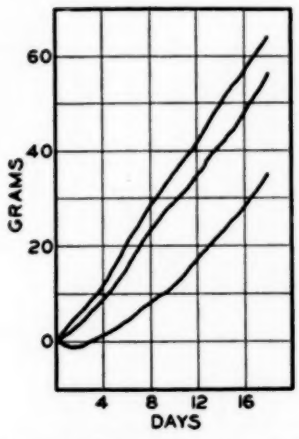

Fig. 4. The ash from wheat gluten is less toxic than the dry gluten.

The upper curve represents the average growth rate of animals receiving a diet containing 15 percent of gluten from normal wheat, while the lower curve represents the growth performance of litter mate animals receiving the same level of toxic gluten. The animals represented by the intermediate curve received ash from toxic gluten equivalent to 15 percent of gluten in the diet.

that the form of selenium in the ash has a less pronounced effect on growth than the form in which it occurs in the plant.

Wheat gluten consists chiefly of the proteins gliadin and glutenin, which are present approximately in about equal proportions. Inasmuch as gliadin can be separated from the glutenin by extraction with warm alcohol, it was of interest to determine whether the selenium would accompany the gliadin. Accordingly, samples of gliadin were prepared from toxic and normal flour. Four hundred grams of flour were extracted with hot 70 percent alcohol. The extracts were reduced to a volume of 400 cc. by distillation under reduced pressure, and the concentrate was then evaporated on ground normal wheat and incorporated in the ration at a level of 12 percent gliadin. The gliadin from the toxic wheat proved to be as toxic as the gluten. Identical results were obtained in a similar experiment in which pure gliadin prepared (see Figure 5) as described by Jones and Wilson (1928) was used.

That wheat plants can absorb selenium from the soil on which they are grown and have toxic properties similar to the grain was demonstrated by feeding wheat plants which Hurd-Karrer of the Bureau of Plant Industry had grown on soil in pots in a greenhouse.

Wheat grown to maturity on soil to which one part per million of selenium had been added was fed, and for control, grain grown under

the same conditions on soil containing no selenium. Both the grain and the straw were used in the feeding experiments. The grain grown on the soil to which selenium had been added was compared in its growth-

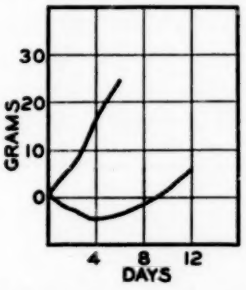

Fig. 5. The gliadin from toxic wheat is as toxic as the gluten.

The upper curve represents the average rate of growth of rats fed a ration containing 12 percent gliadin prepared from gluten from non-toxic wheat. The growth represented by the lower curve resulted when gliadin from toxic gluten was similarly used.

retarding effect with wheat grown in South Dakota and known to be toxic. One group of animals received the basal diet with sodium selenate added to provide 10 parts per million of selenium. Figure 6 shows that

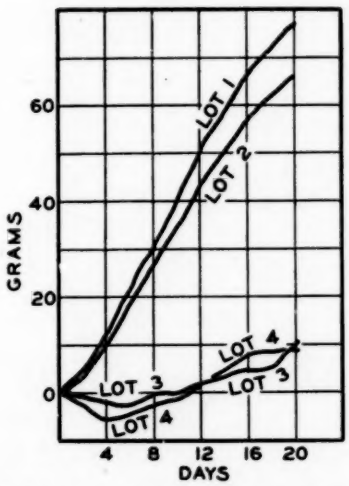

Fig. 6. Toxicity of wheat grown on soil containing selenium.

Lot 1 received wheat grown on control soil, while Lot 4 received wheat grown on similar soil to which sodium selenate had been added in sufficient quantity to make one part of selenium per million part of soil. Lot 3 received toxic wheat grown in South Dakota. Lot 2 received the same ration as Lot 1, except that sodium selenate was added to make 10 parts per million of selenium in the ration.

the wheat grain grown on the soil to which selenium had been added has growth-retarding properties identical with those of toxic wheat grown in South Dakota, while the wheat grown under experimental conditions on soil containing no selenium permitted satisfactory growth. Ten

parts per million of selenium in the diet in the form of sodium selenate had a detectable growth-retarding effect. The toxic effects of this form of selenium becomes much more pronounced if the experimental period is extended.

Because the rat has a rather limited capacity to digest roughages, the straw from the experimental wheat plants was tested on guinea pigs. One control lot of guinea pigs was fed a ration consisting of alfalfa meal 25 percent, normal wheat 25 percent, rolled oats 44 percent, casein

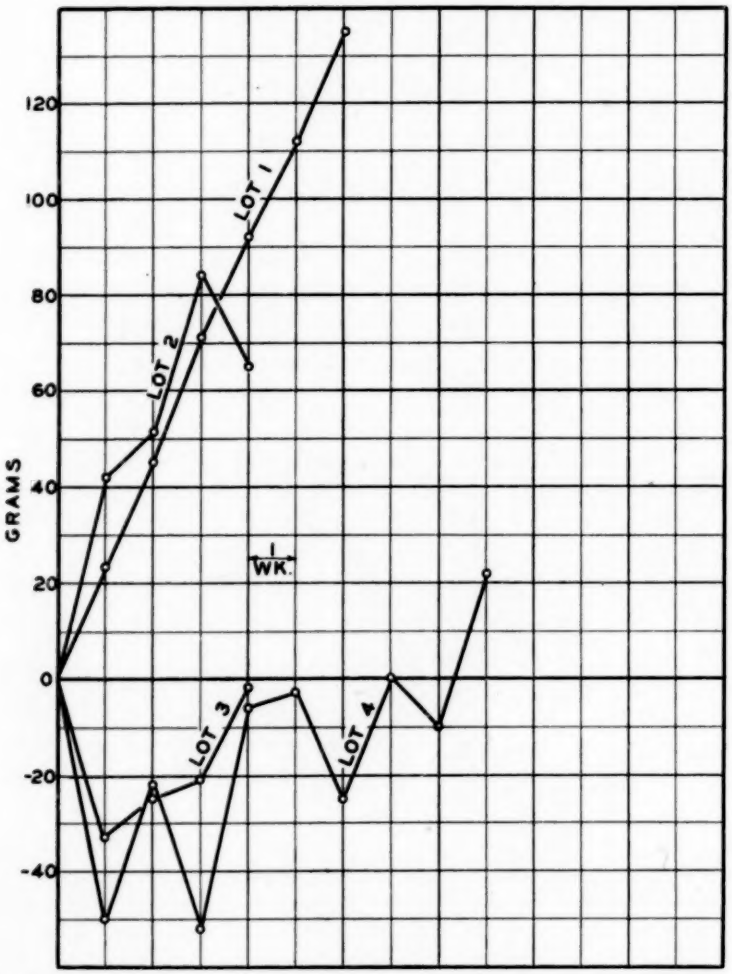

Fig. 7. Average weight changes of guinea pigs fed wheat and wheat straw containing selenium and controls.

The important difference in the rations of guinea pigs whose growth rate is represented by these graphs is that Lot 1 received 20 percent of normal wheat straw, and Lot 2, 20 percent of normal wheat. Lot 3 received 20 percent of wheat straw from plants grown on soil to which sodium selenate had been added, and Lot 4 received 20 percent of toxic wheat grown in South Dakota.

5 percent, and sodium chloride 1 percent. Each animal received in addition 5 grams of fresh cabbage daily. Another control lot received the same ration, except that toxic wheat replaced the normal wheat. The other two lots of guinea pigs received wheat straw in place of alfalfa meal, and oats replaced the wheat in the control rations. The curves in Figure 7 show that toxic wheat and wheat straw grown on a soil to which selenium had been added had the same pronounced growth-retarding effect. The guinea pigs receiving normal wheat or straw in their rations grew at a satisfactory rate.

The results obtained with gluten and gliadin showed that the toxic principle was definitely associated with the protein. We were interested to find out what effect, if any, complete hydrolysis of the protein would have on its toxicity. Quantities of gluten, one prepared from toxic wheat and the other from normal wheat, were hydrolyzed by boiling with sulfuric acid for 36 hours. The sulfuric acid was quantitatively removed, and the hydrolysis products were incorporated in the basal ration in such proportion as to replace the wheat. The growth curves show (Figure 8) that the hydrolysis products possessed the same degree of toxicity as the gluten from which they were formed.

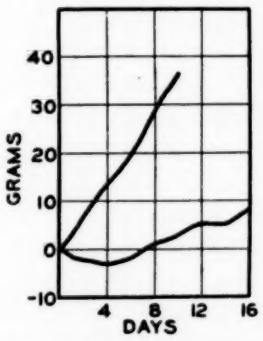

Fig. 8. Average weight changes of rats fed hydrolyzed gluten from normal wheat and toxic wheat. The upper curve represents the average rate of growth of rats fed the hydrolysis products of non-toxic gluten. The lower curve shows the rate of growth when hydrolyzed toxic gluten was used.

Chemical Experiments

The results of the feeding experiments described indicate definitely that the selenium in the toxic grain is present in an organic form intimately associated with or combined with the protein.

One of the most fundamentally important questions of the selenium problem relates to the identification of the organic selenium compound or compounds present in the toxic plant products. Knowledge of the identity and properties of the compound would greatly facilitate the solution of the other problems connected with the subject.

With the view of isolating from the toxic wheat a definite compound containing selenium, gluten prepared from the wheat was hydrolyzed and the selenium compound searched for among the products of hydrolysis, which consist of some 22 or more amino acids. The flour used contained from 15 to 18 parts per million selenium. The gluten was hydrolyzed by boiling with 40 percent sulfuric acid for 36 hours, and the hydrolysis products were subjected to the methods generally followed in protein analysis for the determination of amino acids. This involves first, the separation of groups or fractions of amino acids which have certain properties in common, and then the isolation of the individual members of the groups by fractional crystallization and by reactions characteristic of the individual amino acids.

The dicarboxylic amino acids were first separated from the hydrolysis products by precipitation of their barium salts in aqueous solution by addition of alcohol according to the method described by Jones and Moeller (1928). This group contains glutamic acid, aspartic acid, and hydroxyglutamic acid. This fraction of amino acids from the toxic gluten was incorporated in the basal ration at a level approximately equivalent to that at which these amino acids are present in gluten. When fed to rats there was no indication of the presence of the toxic factor. The absence of selenium in the dicarboxylic amino acid fraction of gluten from toxic wheat has been later repeatedly confirmed in our laboratory by means of chemical tests. Removal from the hydrolysate of this fraction, which represents about 40 percent of the weight of the gluten, effects a great concentration of the selenium.

At the start we were greatly handicapped in our chemical studies because we had no method for following the selenium content in the different fractions separated, except by the feeding method, and by the method of Robinson of isolating and weighing the elemental selenium. The large quantities of material and the length of time required for carrying out these tests made them inapplicable when a large number of small fractions, sometimes weighing only a fraction of a gram, were to be tested. This difficulty was surmounted by a method developed by Horn (1934) consisting of a modification of a test which has been long known and used for detecting the presence of selenium in commercial sulfuric acid. This test involves the use of codeine sulfate whereby a blue color is developed in the presence of selenium. By means of this method the selenium in the small fractions of amino acids can be followed qualitatively, and only a few milligrams of the selenium-containing material are required.

Having demonstrated that the dicarboxylic amino acid fraction could be separated from the hydrolysate of toxic gluten without the removal of any of the selenium, we turned our attention to the fraction commonly

referred to as the hexone bases. This fraction includes the diamino acids, arginine, histidine, lysine, tryptophane, and cystine, which together amount to approximately 10 percent of wheat gluten. This fraction is usually separated by precipitation with phosphotungstic acid. The filtrate remaining after removal of the dicarboxylic amino acids from the hydrolysate was accordingly treated with phosphotungstic acid in the manner usually followed for removal of the hexone bases. The phosphotungstate precipitate was decomposed with barium hydroxide, and the phosphotungstic acid removed. The solution of free bases was then tested for the presence of selenium. Because of the presence of some substance which interfered with the codeine test for selenium, probably traces of phosphotungstic acid, we were unable to show conclusively whether any selenium was present in this fraction. An attempt was then made to effect a separation by extraction of the filtrate from the dicarboxylic amino acids with butyl alcohol according to the method of Dakin (1920). By this method most of the monoamino acids are carried over by the butyl alcohol, while the hexone bases remain quantitatively in the aqueous solution. Examination of the aqueous solution revealed only a trace of selenium. From the results obtained it can be concluded that the fraction of hexone bases in the hydrolysate of toxic gluten carry little, if any, of the selenium. Biological tests for toxicity support this conclusion. Further evidence to support this view has been obtained in connection with extensive direct fractional crystallization of the amino acids present in the hydrolysate remaining after removal of the dicarboxylic amino acids. It should be noted, however, that inasmuch as tryptophane is largely destroyed by acid hydrolysis of proteins, the evidence presented does not apply to this amino acid.

The products remaining in the filtrate from the dicarboxylic amino acids were subjected to systematic fractional crystallization. It was found that most of the selenium could be concentrated in the amino acid fraction which may be referred to as the leucine fraction. This fraction doubtless contained also valine, phenylalanine, and traces of other amino acids. In one experiment, a small fraction was obtained which contained as much as 2 percent of selenium.

The problem of separating from the products of hydrolysis of gluten from toxic wheat a homogenous fraction containing a high percentage of selenium has proved particularly baffling for several reasons. In the first place the amount of the selenium compound present in the wheat flour is very small. The hydrolysate from 2 kilos of the flour contains only about 40 to 50 mg. of selenium as compared with 120 to 160 g. of amino acids. Another difficulty involved is the fact that the selenium compound apparently has properties so similar to those of the amino acids associated with it that we have not yet discovered any property

peculiar enough to it to enable its separation from the accompanying amino acids. We have also been handicapped because of lack of a method for determining quantitatively the amounts of selenium in the large number of small fractions isolated, which can be applied to the small quantities of material available.

From the results thus far obtained the following conclusions can be drawn regarding the nature and properties of the selenium compound in the hydrolysate of the toxic wheat gluten:

The organic selenium compound is quite stable. There are no indications of decomposition when it is heated to boiling with 35 percent sulfuric acid or 20 per cent sodium hydroxide.

It is apparently an organic hydrolytic product of the protein, splitting off in much the same manner as the amino acids. Like the amino acids, the selenium cannot be removed from the unhydrolyzed gluten by water, but after hydrolysis it is readily soluble in water, dilute acids, and alkalies.

In the acid hydrolysate of the toxic gluten it is not associated with the dicarboxylic amino acids, or with the basic amino acids precipitable by phosphotungstic acid, but is carried by the monoamino acid fraction.

It is insoluble in the common organic solvents such as ether, chloroform, carbon disulfide, ethyl acetate, benzene, alcohol, and acetone.

Addition of hydroxyl amine or sodium sulfite to aqueous solutions of the selenium-containing fractions does not throw out selenium, as happens in the case of many inorganic selenium salts.

In general, its properties are very similar to those of the amino acids. It is precipitated along with amino acids when they are thrown down from their solutions as salts of certain metals commonly used for the precipitation of amino acids.

Hydrochloric acid seems to convert the selenium compound into a form soluble in ether. This was observed when hydrochloric acid was used to hydrolyze the gluten. Extraction of the humin with ether removed a small quantity of material containing selenium. When amino acid fractions containing selenium were esterified by heating with hydrochloric acid and alcohol, selenium was found in the ether-soluble part of the esterified product.

By systematic fractional crystallization of the hydrolysis products of toxic wheat gluten, most of the selenium is concentrated in the amino acid fraction which may be referred to as the leucine fraction.

We are not sure whether the compound has an amino and a carboxyl group. In one experiment, working with a small fraction which contained a large amount of selenium, we were able, by condensing it with potassium cyanate in alkaline solution, to obtain a hydantoin in the form of what appeared to be homogenous crystals, which contained selenium.

The formation of hydantoins is a characteristic property of alpha amino acids. However, the reaction was carried out on a small quantity of material, and the experiment should be repeated and confirmed before final conclusions can be safely drawn.

Summary

Chemical and biological evidence is presented showing that toxicity of wheat grown in certain sections of South Dakota is due to the presence of selenium intimately associated with the protein.

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THE MECHANISM OF GAS PRODUCTION IN DOUGH FERMENTATION

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The importance of the phenomenon of carbon-dioxide production in fermenting bakers' doughs is nowadays so well realized that there is no need for any introductory disquisition on the subject. The papers dealing with the matter given in the Index to the first ten volumes of CEREAL CHEMISTRY and the contributions to which these papers make reference, testify to the amount of work which has been done on the subject, but some aspects of the matter have received little attention and it may be of interest to discuss them.

1. The Fermentation of Sugar Solutions

It has been known for many years (see Harden, 1923) that the essential facts of the fermentation of pure sugar solutions by yeast are:

(*a*) That the rate of fermentation is independent of the concentration of the sugar solution between quite wide limits, *viz.*, approximately 0.5% and 20%. The rate increases rapidly up to 0.5% and falls rapidly as the concentration increases beyond 20%, a 50% sugar solution being almost unfermentable. This latter effect is presumably due to plasmolysis of the yeast cells. (See Lafar, 1910.)

(*b*) That in sugar solutions of the same concentration the rate of fermentation varies directly as the yeast concentration.

2. Fermentation in "Liquid" and "Solid" Media

Carbon-dioxide production in a dough must depend essentially on the fermentation of the sugar solution present in the dough, with the difference that in a dough the liquid present is generally supposed to be more or less firmly associated with the starch and gluten, which might be expected to cause essential differences in the manner of fermentation of sugar in ordinary solution and in a dough.

The diagram given as Figure 1 shows the rates of fermentation (1) in plain cane sugar solutions, and (2) in doughs corresponding in all respects to these sugar solutions except that enough pure wheat starch was added to make a firm dough.

It will be seen that affairs differ in the two cases in that (1) in the case of the doughs the rate of fermentation at first increases rapidly for a time, then falls at a steady rate, while the sugar solutions ferment

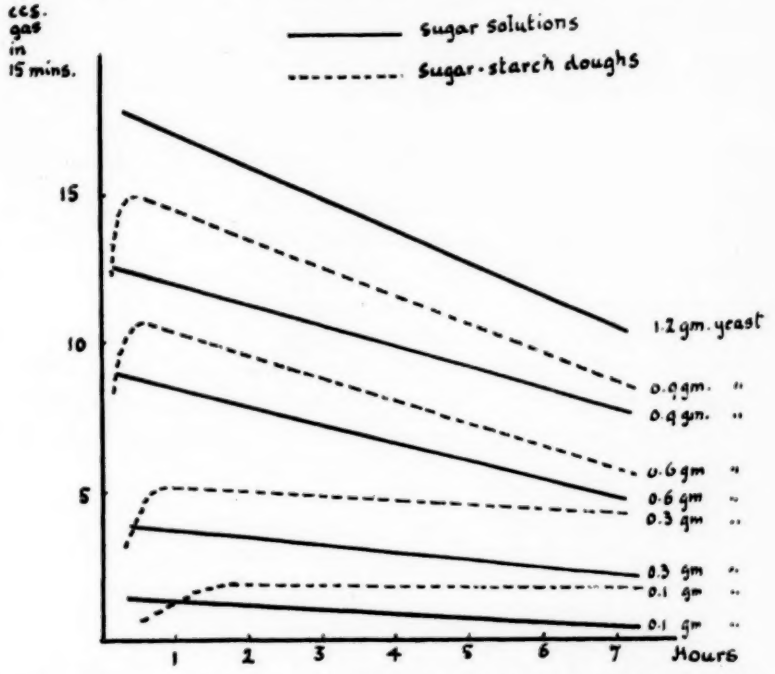

Figure 1. Showing comparative rates of fermentation in sugar solutions and in sugar-starch doughs.

at a constantly falling rate from the commencement, and (2) the rate of fermentation for the same amount of yeast is greater in the case of doughs than in sugar solutions.

A fermenting sugar solution, if left undisturbed, becomes supersaturated with gas to an extraordinary extent, the gas only being given off when the solution is vigorously shaken. In the experiments in question the sugar solutions were only shaken every fifteen minutes, so that possibly the dissolved gas had a retarding effect on fermentation. In fermenting doughs the large surface exposed by the starch granules perhaps prevents this supersaturation.

The initial increase in rate of fermentation, noted in the starch doughs, may be due to the gas at first produced being held in the dough liquid in a state of supersaturation. Exactly the same phenomenon is found in the fermentation of ordinary flour doughs. A dough made up with a low yeast percentage, *e.g.*, 0.25%, may give off no gas for over an hour after being made up, even though the dough be made up with

saturated carbon-dioxide solution. This effect is not due simply to a lag in the inversion of cane sugar, since the addition of dextrose in making up the dough has no effect on the initial lag in gas-making.

The fact that a sugar solution in the form of starch dough ferments as rapidly as a similar plain sugar solution, indicates that as far as fermentation is concerned such a dough acts as a mere mechanical mixture of sugar solution and starch.

3. Growth of Yeast in Fermenting Sugar Solutions

In the fermentation by yeast of a pure sugar solution no growth of yeast can occur. When suitable nutrients are supplied, however, so that growth can occur, the general phenomena of fermentation are quite different from those described in section 1.

To present data on this point experiments were carried out to investigate the rate of gas evolution (1) in plain cane sugar solutions (no yeast nutrient added), and (2) in cane sugar solutions adding suitable yeast nutrients, when fermented with various percentages of bakers' yeast.

The following figures, which were obtained from these tests, refer in all cases to 60 cc. of fermenting solution containing originally 6 g. of cane sugar and (*A*) Hansen's (see Jørgensen, 1925) yeast nutrient containing

0.5 g. peptone
0.15 g. potassium phosphate, K_2HPO_4
0.10 g. magnesium sulphate, $MgSO_4, 7H_2O$

or (*B*) Slator's (1906, 1908) yeast nutrient containing

0.54 g. asparagin
0.18 g. potassium phosphate
0.12 g. magnesium sulphate

In all cases the temperature of fermentation was 30° C. A quantity of glass beads was placed in the fermenting solution, which was vigorously shaken for one minute before each reading was taken. Readings were taken at intervals of fifteen minutes. The data from these experiments are recorded in Tables I to IV inclusive.

In all the experiments described in this paper, the sugar solutions and doughs were made up with water saturated with carbon-dioxide. The only variable in all these experiments was the amount of yeast originally added, and in experiments 16 and 17, where the amount of nutrient added was respectively double and quadruple that used in experiment 15.

The yeast cells in the suspensions were counted, after appropriate dilution if necessary, by means of the haemacytometer.

TABLE I

GROWTH OF YEAST IN FERMENTING SUGAR SOLUTIONS

A. Hansen's Yeast Nutrient

Experiment number	Original yeast	Yeast cells per standard volume			Total time of fermentation	Rate of gas production			
						No nutrient		With nutrient	
		Original	Final—no nutrient	Final—with nutrient		1st hour	4th hour	1st hour	4th hour
	%				*Hours*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
10a	0.17	8.8	8.4	12.4	4	6	4.5	6.5	16.5
12	1.00	50.0	45.0	55.0	4	34	28.5	39.0	59.0
13	3.00	172.0	173.0	173.0	4	104	98.0	126.0	132.0
14	6.00	333.0	320.0	330.0	4	212	180.0	250.0	230.0
15	8.00	424.0	420.0	420.0	4	280	240.0	326.0	310.0
16	8.00	—	—	403.0	4	280	244.0	350.0	310.0
17	8.00	—	—	430.0	4	—	—	342.0	330.0

TABLE II

GROWTH OF YEAST IN FERMENTING SUGAR SOLUTIONS

B. Slator's Yeast Nutrient

Experiment number	Original yeast	Yeast cells per standard volume		Total time of fermentation	Rate of gas production			
					No nutrient		With Nutrient	
		Original	Final—with nutrient		1st hour	last hour	1st hour	last hour
	%			*Hours*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
18	0.5	24	46	7	13	12	17	80
19	1.0	46	70	7	30	22	38	99
20	2.0	94	117	$5\frac{1}{2}$	70	54	84	138
20a	3.0	129	134	4	100	84	126	166
21	4.0	170	180	4	138	112	180	200
22	6.0	238	227	4	204	182	258	258

Dividing the increase in the number of yeast cells in the nutrient solutions at the end of fermentation, by the number present in the original solution and expressing the result as a percentage

$$\left(\frac{\text{final} - \text{original}}{\text{original}} \times 100\right),$$

gives a measure of the growth of yeast, while similarly, the figures for gas produced in the first and last hours of fermentation can be made to give a measure of the acceleration of the gas production.

TABLE III

A. HANSEN NUTRIENT

Experiment number	Original yeast	Growth of yeast	Acceleration in gassing
	%	%	
10a	0.17	41	152.0
12	1.00	10	51.0
13	3.00	nil	5.5
14	6.00	nil	nil
15	8.00	nil	nil
16	8.00	nil	nil
17	8.00	nil	nil

TABLE IV

B. SLATOR'S NUTRIENT

Experiment number	Original yeast	Growth of yeast	Acceleration in gassing
	%	%	
18	0.5	92.0	370
19	1.0	52.0	160
20	2.0	24.5	64
20a	3.0	4.0	32
21	4.0	6.0	11
22	6.0	nil	nil

It is clear that yeast when in active growth ferments sugar much more rapidly than when little or no growth is occurring, the proportional increase in rate of fermentation being much greater than the proportional growth of yeast; also that growth of yeast is greater the lower the original yeast concentration, and ceases when the original concentration is somewhere about 130 to 170 cells per standard volume.

The figures also show that with nutrient present, even when little or no yeast growth occurs, the rate of gas production is greater than when no nutrient is present, and further, that the limit of growth of yeast cells with its accompanying lack of acceleration in the rate of fermentation, is not simply due to lack of nutrient, since doubling or quadrupling the amount of nutrient, as in experiments 16 and 17, does not cause any appreciable growth of yeast, nor affect the rate of fermentation.

The type of nutrient used affects the results, Slator's nutrient being more efficient than Hansen's.

These results are in agreement with those obtained by Brown (1892) who investigated the phenomena of growth of brewers' yeast in brewers' wort in relation to the fermentative activity of the yeast, measuring either the sugar destroyed or the alcohol formed, and his experiments related to longer periods, generally 12 or 24 hours. Brown also found

that brewers' yeast in a state of active growth fermented sugar much more rapidly than when no growth occurred, and further, that given sufficient time yeast growth went on until a certain limiting concentration of yeast cells was reached, provided naturally that the original concentration was below that limit. This last conclusion had previously been reached by other workers. (See Lafar, 1910, p. 231.)

In these experiments Brown found this limiting concentration to be about 26 yeast cells per standard volume, whereas in the experiments just described the limiting concentration of yeast cells at which no growth occurs, is about 150 cells per standard volume. This difference may be connected with the fact that modern bakers' yeast is of a different type from English brewers' yeast, being of a much " faster " character.

In later experiments Brown (1905) found that yeast cell reproduction under anaerobic conditions—the conditions, that is, of his earlier experiments—is governed by the amount of oxygen originally at the disposal of the cells prior to commencement of reproduction, and that in the presence of a continual supply of oxygen the extent to which yeast cells can grow depends on the supply of nutrients.

4. Fermentation and Growth of Yeast in Doughs

The following are the rates of gas evolution per hour from doughs made up in all cases from 20 g. flour and 12 cc. liquor, fermented at 30° C., and containing different amounts of yeast; fermentation was continued for 8 hours, readings of gas evolved being taken every 15 minutes.

TABLE V

FERMENTATION AND GROWTH OF YEAST IN DOUGHS

Yeast on flour	Yeast used	Rate of gas evolution per hour								Maximum rate
		1	2	3	4	5	6	7	8	
%	*Grams*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
0.25	0.05	1	1	6	10	17	23	28	30	30
0.50	0.10	3	8	13	19	26	31	32	34	34
1.00	0.20	6	18	25	32	35	36	33	16	36
1.50	0.30	12	28	34	39	42	34	16	12	42
2.00	0.40	14	36	39	44	40	22	13	11	44
2.50	0.50	17	43	45	49	27	16	13	10	49

Such figures are typical. The following are the maximum rates per hour for four different flours, the experiments being conducted in a manner similar to that already described, except that fermentation was continued for 5 hours.

TABLE VI

FERMENTATION AND GROWTH OF YEAST IN DOUGHS

Yeast on flour	Yeast used	Maximum rate per hour			
		Flour			
		1	2	3	4
%	*Grams*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
0.5	0.1	32	34	44	52
1.0	0.2	44	42	58	64
2.0	0.4	51	54	70	82
3.0	0.6	64	64	80	90
4.0	0.8	72	74	96	102
5.0	1.0	72	82	98	116

In Table V it is seen that the rate of gas evolution rises very definitely as fermentation progresses, this rise being the more marked the lower the original percentage of yeast used, and in Tables V and VI that the maximum rate attained is proportionately much higher the lower the original percentage of yeast. Such figures suggest that growth of yeast is actually occurring in the doughs, that the lower the original percentage of yeast used the greater the growth, and that the general phenomena may be the same as when sugar solutions containing yeast nutrients are fermented.

The method described by the writer (1936) for counting yeast cells in dough, based on that of Turley (1924), was applied to investigate this suggestion. The data are shown in Tables VII, VIII, and IX.

Doughs from 20 g. of four different flours were made up with various percentages of yeast and fermented for 5 to 6 hours, readings of gas evolution being taken every 10 or 15 minutes. Each dough was given 1.25% salt, calculated on the flour used. At the end of the fermentation period the dough was digested with pepsin HCl, and with diastatic malt extract, as described, and the yeast cells counted. The yeast cells in the original suspension used for making up the dough were also counted. In each case the original yeast suspension used for making up the dough was diluted so as to give a 0.01% yeast suspension, and the cells in 144 small squares (each equivalent to one 400th cu. mm.) were counted in a Leitz haemacytometer. The corresponding doughs after digestion were similarly diluted, the cells in 144 small squares in 8 slides being counted.

Thus in the case of the experiments using 0.25% yeast calculated on the flour, the yeast suspension contained 0.5% yeast, 20 g. of each of the four flours being made into a dough by adding 10 cc. of the yeast suspension; 10 cc. of the 0.5% suspension were made up to 500 cc.,

giving a 0.01% suspension. The doughs after fermentation and digestion were made up to 500 cc.

In the case of the experiments using 4% yeast calculated on the flour, an 8% yeast suspension was made up, 10 cc. of this was made up to 500 cc. and 25 cc. of this diluted suspension made up to 400 cc., the concentration of yeast in this second dilution being again 0.01%. The liquor obtained after digestion of the fermented dough was made up to 500 cc., and 25 cc. of this diluted to 400 cc., and so on.

TABLE VII

ACTUAL AVERAGE COUNTS OF YEAST CELLS OBTAINED IN 144 SMALL SQUARES [1]

Yeast on flour	Yeast cell count—original suspension	Yeast cell count—after fermentation			
		Flour			
		1	2	3	4
%					
0.25	65 : 67 Average....66	153	147	167	142
0.5	62 : 70 " 66	140	148	125	137
1.0	63 : 65 " 64	108	105	94	114
2.0	62 : 61 " 61	75	63	55	74
3.0	72 : 67 " 69	65	60	63	72
4.0	60 : 63 " 61	62	56	51	63

[1] From such figures the equivalent percentage of yeast present at the end of fermentation can be calculated.

TABLE VIII

ORIGINAL QUANTITY OF YEAST USED AND THE CALCULATED QUANTITY OF YEAST FINALLY PRESENT; ALSO THE INCREASE IN YEAST CONCENTRATION, THE ORIGINAL CONCENTRATION IN EACH CASE BEING TAKEN AS 100

Original yeast present	Original quantity of yeast used	Calculated quantity of yeast finally present				Growth of yeast			
		Flour				Flour			
		1	2	3	4	1	2	3	4
%	*Grams*								
0.25	0.05	0.12	0.11	0.13	0.11	132	124	152	116
0.50	0.10	0.21	0.22	0.19	0.21	112	124	90	108
1.0	0.20	0.34	0.33	0.29	0.36	69	64	47	78
2.0	0.40	0.49	0.41	0.36	0.48	23	3	−10	21
3.0	0.60	0.57	0.52	0.54	0.63	−6	−13	−10	4
4.0	0.80	0.81	0.74	0.67	0.79	−1	− 8	−16	−2

It is clear from these figures that when relatively low percentages of yeast are used in making up the doughs, growth of yeast does actually occur and to a very definite extent considering the short fermentation periods involved; but also there is a limit corresponding to roughly 2%

of yeast calculated on the flour used, at and above which no growth occurs.

TABLE IX

MAXIMUM RATES OF GAS EVOLUTION PER HOUR, AND THE CALCULATED MAXIMUM RATES PER 1 GRAM YEAST FINALLY PRESENT

Original yeast present	Maximum rate of gas evolution per hour							
	Actual				Per gram yeast finally present			
	Flour				Flour			
	1	2	3	4	1	2	3	4
%	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
0.25	27.5	27.5	29	33	229	250	227	300
0.5	33.0	35.0	36	44	157	159	189	209
1.0	47.5	47.0	51	55	140	142	176	153
2.0	52.0	52.0	51	59	106	127	142	124
3.0	70.0	71.0	72	78	134	137	133	124
4.0	78.0	79.5	86	94	96	107	128	119

In the light of Brown's (1905) later experiments we may expect that this limiting concentration of yeast cells in a dough will depend on the amount of oxygen dissolved in the dough liquor when the dough is first made up, and possibly that the rate of fermentation will depend on this oxygen concentration also.

Brown's conclusions may support the commercial bakers' view that the common practice of giving a dough a thorough "cut-back" or remixing a while before the dough is scaled off, has the effect of refreshing the fermentation by giving the yeast cells a fresh supply of atmospheric oxygen.

The references in the literature to the possibility of growth of yeast occurring in dough fermentation are very sparse. Jago (1895) states dogmatically that "functioning in dough, no reproduction of the yeast occurs," further, on the basis of experiments of which, however, no details are given, he found that in a very slack sponge no reproduction of yeast occurs. Jago also quotes Briant as stating in the Bakers' Quarterly Trade Review that reproduction of yeast may occur in a ferment provided the latter is not too concentrated, but no exact details of this reference are given.

Bennion (1929) states that "research . . . seems to show that yeast does reproduce in a dough, but slowly, because of the density of the medium, and lack of oxygen." Also, "yeast is not able to work without reproducing, so that while the density of the medium may effect (? affect) the rate of reproduction, some reproduction certainly does take place." These statements the present writer is unable to confirm.

Under average British baking conditions less than 1% yeast reckoned on the flour, is used. What is known as the "4-hour-straight-dough" process—a very common process—uses only about 0.6% yeast, while the "all-night" straight-dough process requires only about 0.2% yeast. American bread-making processes apparently require more yeast, about 1¼% according to Steen (1935). But it seems clear that the average amount of yeast used in normal bakery practice in America, and still more in the United Kingdom, is such that growth of yeast certainly takes place.

It is clear too that with these relatively low original percentages of yeast, when growth of yeast is occurring the maximum rate of gas evolution attained is higher than with higher original yeast percentages, when no growth occurs, so that exactly as was found with fermenting sugar solutions containing yeast nutrients, growing yeast in a dough ferments the saccharine pabulum present at a much greater rate than is accounted for by the mere growth of the yeast.

Those doughs containing 2% yeast, calculated on the flour, were made up using 10 cc. 4% yeast suspension for 20 g. flour, and the 4% yeast suspension was found to contain yeast cells at the rate of 170 per standard volume, a figure corresponding sufficiently well with the figure of 150 cells per standard volume already mentioned as being the limiting concentration of yeast cells in a fermenting sugar solution containing yeast nutrient, at which yeast ceases to grow.

To determine whether this limiting concentration of yeast cells is controlled by lack of yeast nutrients, doughs were made up as follows from a flour of low-gas-making capacity, and the gas production measured as in the foregoing experiments. The yeast count in the doughs after 5½ hours fermentation, and in the original yeast suspension used, was determined.

Dough Characteristics

1. No added nutrient
2. 0.3% diastatic malt flour added (120° Lintner)
3. 0.1% ammonium acid phosphate added
4. 0.3% diastatic malt flour and
   0.1% ammonium acid phosphate added

	No. 1	No. 2	No. 3	No. 4
Percentage growth of yeast	24	20	30	24
Maximum rate of gassing per hour, *cc.*	56	60	56	69
Total gas evolved after 5½ hours, *cc.*	175	256	179	269
Time after which rate of evolution began to fall, *minutes*	155	221	152	258

The " nutrients " did not cause any greater growth of yeast than occurred in their absence, *i.e.*, the limiting concentration effect already discussed is purely a concentration effect, and has nothing to do with the supply of the nutrients used, at any rate in this particular experiment, just as was found in the fermenting sugar solutions given extra doses of yeast-nutrient (see 15, 16, 17, Tables I and II). The effect of the ammonium phosphate added was nil in any respect. Diastatic malt flour, and still more the combination with ammonium phosphate, increased the maximum rate of fermentation to some extent, and, still more important, prolonged the time elapsing before the rate of gassing began to fall.

Calculating the maximum rate of gas evolution per hour, per gram yeast present at the end of the fermentation period, in the case of the cane sugar solution with Slator's nutrient, and in the case of the four flours Nos. 1 to 4, gave the following comparison—

Yeast in suspension %	Sugar solution	Flour dough
0.5	138	251
1.0	109	179
2.0	99	153
4.0	79	125
6.0	75	132

The corresponding figures for rate of gas production are of the same order of magnitude, but higher for the fermenting dough in each case (to the extent of about 75%). The general state of affairs is reminiscent of the comparison between the fermentation of plain cane sugar solutions and that of the same solutions made into a dough by adding starch, as described above.

It is customary to talk of the moisture added in making up a flour-dough as existing in some sort of colloidal absorption or combination with the flour solids. In such a case it is difficult to conceive how the phenomena of gas production, which have been shown to be so closely concerned with the concentration of yeast cells in the fermenting medium, are essentially the same in a dough as in a nutrient sugar solution. Any marked absorption of water as such from the dough-liquid by the flour solids, would increase the concentration of the yeast cells, causing conditions which tend to inhibit rather than stimulate gas production, and the growth of yeast.

The exact nature of the nitrogenous and mineral matters which must in some way be available for the growth of yeast in a dough, and the manner in which they are provided, is a subject on which little information is available. The hydrolysis of phytin by the phytase present in the flour will provide the calcium, magnesium and phosphorus,

for instance, required for growth, and it is possible that the rate at which the various nutrients required are made available during fermentation may therefore have some controlling effect on the rate of fermentation, adding a further complication to a process which it will be appreciated is anything but simple.

To the writer it appears that however desirable it may be to evolve rapid and simple laboratory methods for determining the factors on which gas production in dough fermentation depends, such as the production of maltose by diastatic action during the latter part of fermentation, it is essential to carry out gas production tests reproducing as closely as ever possible the actual conditions under which flours will be used in commercial practice, if a true picture of the gas production process is to be obtained, a remark which applies particularly to the routine testing of commercial flours in the laboratories of flour mills and bakeries. Such determinations of gas production are easily made, using quite simple, inexpensive apparatus. Various devices have recently been described by which the course of gas production can be mechanically recorded. (See Markley and Bailey, 1932.)

Quotations from a paper by Kozmin (1933) add point to these statements. Mme. Kozmin says with respect to measurement of fermentation rate in her experiments, that using quantities of yeast representing "the maximum quantity of yeast used under *our conditions* (Mme. Kozmin's italics) in commercial straight doughs" . . . "it is quite clear that notwithstanding a very considerable variation in the diastatic activity of the flour samples, their rate of fermentation was practically the same." This conclusion follows naturally from the consideration that in a fermenting sugar solution the rate of fermentation, other things being equal, depends *only* on the quantity of yeast present, provided that the concentration of fermentable sugar does not fall below about 0.5%, and is constant between quite wide limits of sugar concentration. What the diastatic activity of a flour does govern is the *time* for which the maximum rate of fermentation lasts, the time, namely, during which the pre-existing cane sugar and the maltose formed by diastatic action together can keep the concentration of the sugar solution in the dough above that limit of about 0.5%. Diastatic activity has but little effect on the rate of fermentation in the earlier stages.

It is well enough understood nowadays that the real significance of gas production in a fermenting flour dough is the ability of the various factors involved to maintain gas production *during the proving stage* at a sufficiently high level, and the question of the actual mechanism of gas production in these later stages is one presenting some curious features which it is hoped to discuss at a later date.

Summary

In a fermenting flour-dough the rate of fermentation is profoundly influenced by the fact that growth of yeast may occur.

The extent to which yeast grows is controlled by the amount of yeast used in making up the dough, being greater the less the amount of yeast used.

When the amount of yeast used is about 2%, or over, calculated on the flour, no growth occurs.

The rate of gas evolution from a dough increases steadily as fermentation progresses, the increase being the greater the lower the original percentage of yeast used.

The more rapidly yeast grows in a dough, the more rapid the rate of gas evolution in proportion to the actual amount of yeast present.

The general phenomena of gas evolution in fermenting doughs are essentially the same as in fermenting sugar solutions supplied with yeast nutrients.

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CHANGES IN ENZYMES DURING MALTING[1, 2]

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Introduction

One of the principal aims of malting technology is so to utilize the vitality manifested in the barley grain during the malting process, that the highest possible concentration of enzymes is procured, in order that the insoluble food material deposited in the grain during ripening may be subsequently obtained in soluble form in the extract during the mashing process. Efforts toward this end are complicated by the economically important problem of obtaining the requisite enzyme concentration with the minimum of respiration loss. The practical maltster determines the results of his malting procedure empirically, by noting the degree of solution of the material formed in the grain. The analytical chemist, in place of this frequently deceptive empirical observation, is able to obtain a reliable estimate of the degree of solution by a differentiation of the organic nitrogen of the malt or by a determination of several enzymic activities. Such data provide at once the necessary estimate of value at the time of observation. Only a more comprehensive insight into the chemical nature of the malt will eventually allow us to give an estimate as to its apparent suitability for its intended purpose and for the commercial mashing process. My address will be confined to an exposition of the development of the most important enzyme activities during the malting process.

Carbohydrate Splitting Enzymes

I shall first take up the group of carbohydrases, the carbohydrate splitting enzymes, of which amylase has been most extensively studied and which demands primary consideration both from a qualitative and quantitative standpoint. The relatively simple and apparently obvious relationships offered earlier appear to be much more complicated today since recent researches have provided a more comprehensive insight into the nature of amylase and methods have been found to measure quantitatively its separate functions. According to Ohlsson (1930) and collaborators, the following functions of amylase may be distinguished: (1) Saccharogenamylase, the enzyme component of the unsprouted grain. To it is due substantially the sugar forming activity of amylase. By Kuhn's (1925) nomenclature it is a beta-amylase, and is characterised

[1] Printed in German in Wochenschrift für Brauerei 52: 249 (August, 1935).
[2] Read in French at the Fourth International Congress of Agricultural Chemistry in Brussels, 1935.

by its sensitivity to temperatures above 60° C., its stability toward acidity to a pH of 3.3. (2) The dextrinogenamylase, an alpha-amylase. This is a typical component of germinated barley, becoming strongly active after three to four days of malting and is characterised by its great lability toward acid and its remarkable stability toward temperatures above 60° C. It produces an extensive cission of the molecular linkages in starch, rapidly breaking the large molecule into many smaller aggregates as shown by Waldschmidt-Leitz (1933), and thus creates many points of attack for the saccharogenamylase. It is an open question as to whether the liquefying power of amylase is an independent enzyme or is associated with the two components. Myrbäck and Myrbäck (1932b) conclude from theoretical considerations that no particular starch liquefying enzyme need be assumed but that liquefaction of the starch is a direct consequence of the breaking of glucosidic linkages by alpha- and beta-amylase. Many observations by Chrzaszcz (1933) and collaborators, as well as by Lüers and Rümmler (1933, 1935), indicate that while a close connection exists between the liquefying power and the dextrinogen-amylase, the two enzymic functions do not seem to be identical.

A further problem pertinent to the discussion is the nature of the development of amylase during germination. The earlier view held was that a systhesis of the enzymes occurred during the course of germination (see Hesse, 1920, 1934). Today however we incline to the belief that sprouting induces, in part, a liberation of preexisting but inactive amylase present in the grain, and, in part, a development of activating substances. Of significance for the change in our concepts are the researches of Ford and Guthrie (1908), Jozsa and Gore (1930, 1932), Weichherz and Asmus (1931), as well as Lüers and Lechner (1933) on the liberation of amylase by digestion with papain. Likewise those by S. Nishimura (1928), Waldschmidt-Leitz and Purr (1931, 1932) on amylase activators, and finally the interesting work of Chrzaszcz and Janicki (1933) according to which amylose in the granule is accompanied by specific inhibitory substances (sistoamylase) for which there are specific antibodies (eleutoamylase). These authors also believe that they have found a typical kinase for dextrinization in trypsin preparations. Since protein degradation products play a significant part in the liberation of amylolytic activity as well as for its activation, it may be assumed, as previously developed by Waldschmidt-Leitz (1931, 1932) that the appearance of the amylase function is dependent upon the preceding strongly active development of proteolysis.

Since the theory may appear to be incomplete and contradictory, let us consider a quantitative observation of the phenomenon of the devel-

opment of amylase during germination. The following figure taken from the work of Lüers and Rümmler (1933, 1935) illustrates the results in a simple and comprehensive manner.

Figure 1 shows the development of sugar forming activity during malting expressed in grams of maltose per 100 grams of dry matter.

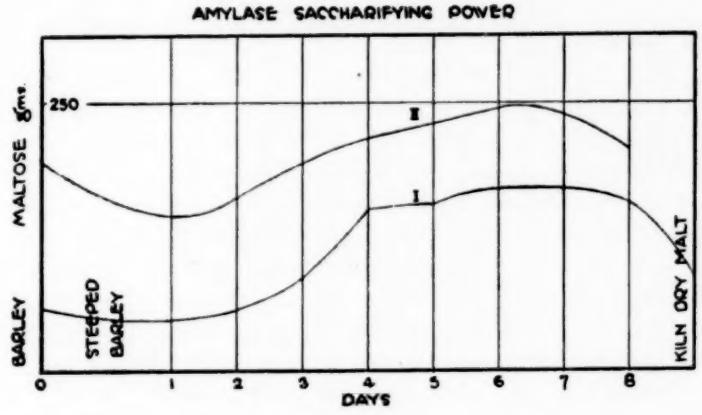

Fig. 1.

Curve 1 gives the value of the normal extract, curve 2 that of an extract after digestion of the same sample with 1% papain for 23 hours at 26° C. It is obvious that by digestion with papain a considerable amount of amylase is liberated. The difference diminishes with continued germination and finally according to Weichherz and Asmus (1931) disappears after ten to fourteen days germination.

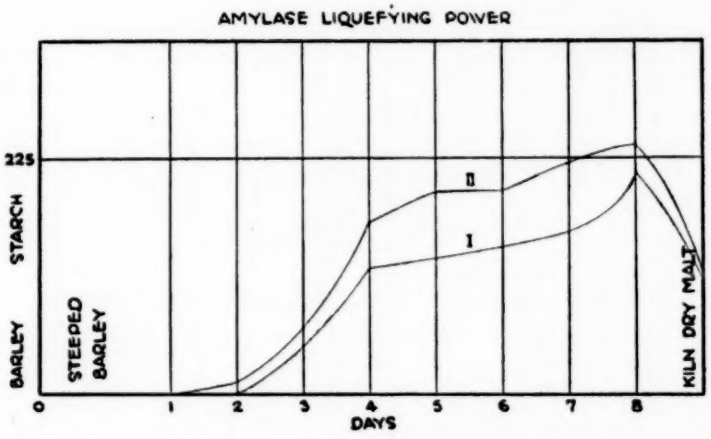

Fig. 2.

Figure 2 represents the development of the liquefying activity of the same malt expressed in grams of starch per gram of dry substance according to the Lintner-Sollied-Doesel method (see Pawlowski, 1927); also with and without papain digestion. Even though this method may not be highly sensitive the enormous increase of this component during three to four days germination is obvious and is so extraordinary that one can hardly refrain from the impression that an actual enzyme synthesis occurs.

Figure 3 represents the development of the dextrinizing activity of the same malt determined according to the reaction velocity method of Sabalitschka and Weidlich (1929). The curve is so similar to that of the liquefying activity that one feels justified in assuming a close connection between the two functions; but that they are still not identical was shown by Lüers and Rümmler (1933, 1935), who destroyed alternately the sacchro- and dextrinogenamylase according to Ohlsson (1930) and then determined quantitatively the single functions. It was thus found, for example, that the liquefying power lost at most 10% of its activity under the influence of temperature, while the dextrinizing activity fell to 65% of its original value. Moreover, although the two enzyme functions react similarly to higher acidity and inactivation by ultra-violet light, there are important quantitative differences.

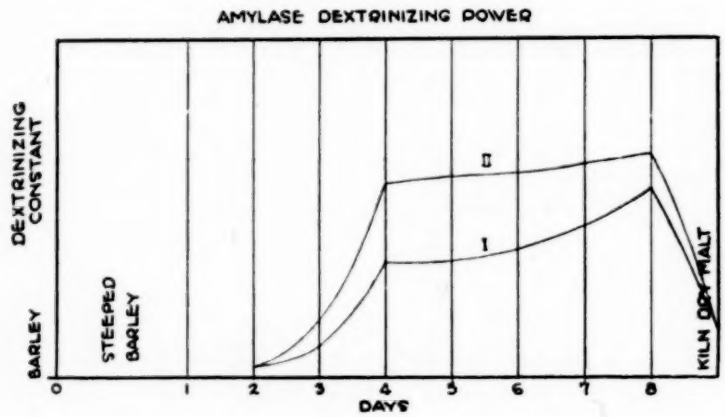

Fig. 3.

Again the amount of amylase in malt is of technological importance. In general, it can be ascertained that small-grained, high-protein barleys give a higher diastatic activity, under identical malting conditions, than large-grained, low-protein barleys. The distillers, for whom the malt is essentially only a source of diastase, make good use of this fact since they employ the light barleys which are not considered useful in brew-

ing. Moreover, it has been established that a cool malting always produces a higher diastatic activity than a warmer one. Finally, according to experiments of Effront (1905, 1906), the length of the malting has a considerable influence. While the liquefying power continually increases during germination up to 25 days and then remains constant, the saccharifying power reaches its maximum in 20 to 23 days and then slowly diminishes. Lastly, the physiological relationships of each crop year play a not inconsiderable rôle, as has been shown particularly by Bach and Oparin (1922, 1922a). Many crop years are abnormal, the malt lacks in diastatic activity, and causes trouble with saccharification in the mash house. As Lüers and Wieninger (1929) have shown in a number of such cases the principle cause was a deficiency in the formation of liquefying activity in the normal saccharification.

With regard to the influence of drying, I may confine myself to a reference to the fact that the diminution in enzyme content, always observed upon drying, is controlled by the temperature, time and water content of the malt, and consequently can become quite important in the case of dark malts of the Munchen type, but is often inconsiderable in the case of the bright Pilsner type.

We shall consider the remaining carbohydrate splitting enzymes very briefly because of their small practical importance and further because our knowledge regarding them is insufficient. The alpha-glucosidase, which splits maltose into two molecules of glucose, has repeatedly been shown to be present in malt and barley by Ling and Nanji (1923), Pringsheim and Leibowitz (1925), and others. (See also the literature under Hesse, 1920, 1934.) Loibl (1923) followed the development of the enzyme during malting and found it, as illustrated in Figure 4, to double in concentration from barley to green malt while in the withered malt just before kilning the increase was three times the original. Here, also, drying produced a considerable decrease. It can thus be concluded that in commercial starch degradation small amounts of glucose are formed along with maltose by the maltase.

Likewise, the researches of Pringsheim and Genin (1924) and Pringsheim and Baur (1928) give proof of the presence of beta-glucosidase, since cellobiose was split by addition of malt extract. According to von Laer (1921, 1921a) extracts of green and dry malt split amygdalin and salicin, which is further evidence for the presence of beta-glucosidase activity.

Lastly, among the simple carbohydrates, the existence of beta-*h*-fructosidase (invertase) in barley and malt has been shown (see O'Sullivan, 1900, and Maestrini, 1921), as is to be expected, since sucrose is an important constituent of malt. It is surprising, however, that there

are no observations concerning the change in invertase from barley to malt.

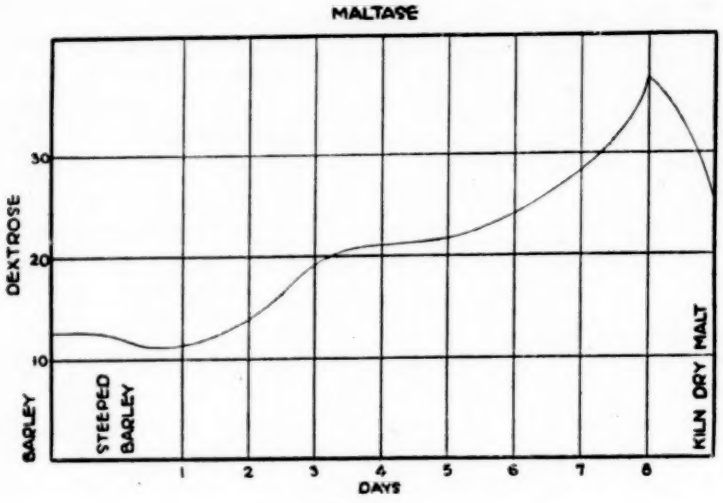

Fig. 4.

Our knowledge regarding the existence in malt of those enzymes which hydrolyze the higher polysaccharides is even more scanty and incomplete, particularly those which attack the tissue and cell wall constituents and which are concerned with the solution of the endosperm. A great deal of contradiction and uncertainty exists in this field due for the most part to the fact that we still know so little about the particular chemical structure of the cell walls. What we observe in a single case is for the most part a vaguely defined portion of the process comprising "cytolysis."

According to the observations of Maestrini (1921b) and Loibl (1923) an attack of the real natural cellulose of the barley during germination does not appear probable. Cellulose, not natural to the grain and its degradation products, however, appears to be susceptible to splitting by malt extract according to Pringsheim and Baur (1928). Moreover, lichenin, a reserve food, is split by addition of malt-producing cellobiose according to Pringsheim and Seifert (1923), and Karrer, Straub, Weinhagen, and Joos (1924). Karrer and Straub (1924) also studied the development of lichenase during germination and found an increase in the enzyme content of about 50% after eight to eleven days growth of the barley.

Mannans, polysaccharides of mannose, are split by the addition of malt, see Effront (1897), and Bourquelot and Hérissey (1899, 1900). In addition Pringsheim and Genin (1924) report an enzymic hydrolysis of salepmannan by malt extract.

The pentosan or hemicellulose splitting enzymes are by far the most difficult to evaluate since here the lack of our knowledge regarding the constitution of these chemical individuals, in addition to the inadequacy of the analytical methods, present singular difficulties. But cytolysis surely plays a decisive rôle in this group of carbohydrates which contribute to the structure of the cell wall. Simple and exactly defined substrates are obtainable only in the natural condition. Thus Karrer and Ehrenstein (1926) obtained a splitting of xylan to xylose by the addition of malt, the xylan being prepared from straw by the method of Salkowsky-Hëuser (see Hëuser, 1921). Loibl (1923) showed that during germination an extraordinary increase (about five times) in soluble pentosans occurred as determined by the method of Kröber-Tollens (see Kröber, 1901), (see Figure 5).

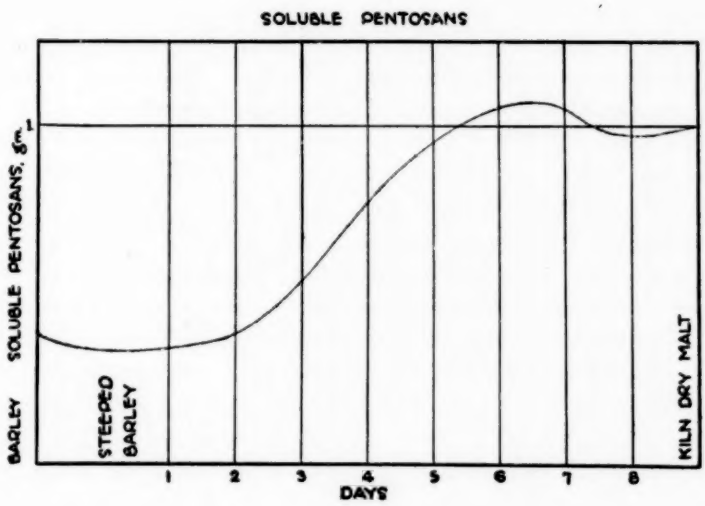

Fig. 5.

Since polygalacturonic acids and polyglucouronic acids also form furfural on distillation with hydrochloric acid, Hesse (1920, 1934), particularly, surmises that we are here concerned with only a pentosan degradation (by chemical means). Even if, on the basis of this newer knowledge, the extraordinary increase in soluble pentosans is not proved, the conclusion must still finally be drawn that one of the constituents of the cell membrane is subject to hydrolysis during germination. Lastly, the presence in malt of enzymes which split pectinous material has been demonstrated by Bourquelot (1898), Carré (1922), and Ehrlich (1927). The barley gum, galaktoxylan, first prepared by Lintner

(1890) has been definitely shown to be subject to enzymic splitting by Lüers and Fries in still unpublished work. In view of the great importance of cytolysis, it is to be hoped that intensive work will soon result in greater clarification.

Proteases

We shall now leave the carbohydrases and turn to the proteases. Their importance is at least as great, if not greater, than that of the carbohydrases since upon the correct degradation of the malt proteins depend the properties of the beer, such as body, head, sediment, turbidity, *etc.* Science has been greatly concerned with the degradation of proteins during malting, but only the most recent researches have clarified the frequently perplexing views of the earlier workers, thanks to the progress of our knowledge and the refinement of our analytical technique and methods of preparation, principally through the school of Willstätter (1928). I may, therefore, neglect the older literature as it is exhaustively reviewed by Hesse (1920). According to the important work of Lundin (1922), and independently of Lüers and Malsch (1929), Mill and Linderström-Lang (1929), Hopkins (1929, 1930), von Euler, Myrbäck and Myrbäck (1929), and Ambros and Harteneck (1929) it can be demonstrated that there exists in malt a protease with a pH optimum of about 5 and in addition at least two different peptidases. The proteinase of barley and malt is of the papain type and can be activated by hydrocyanic acid. The optimum activity of the peptidases is at neutral or slightly acid reaction. Interesting studies concerning the method of separating these peptidases in the germinating grain are described by Linderström-Lang and Holter (1931, 1932).

The analytical determination of these enzymes is made by the use of certain substrates such as gelatin, edestin, or different dipeptides and constitutes the foundation of our scientific knowledge. For practical interpretation, however, these methods are not suitable. In this connection, the natural substrate, or still better the whole system, the germinating grain itself must be considered and the attempt made to obtain an insight into the proteolysis by means of convenient and practical methods of differentiating organically combined nitrogen, which will permit such an estimation to be made. Unfortunately the extraordinarily tedious methods of Schjerning (1900) are unsuited to the requirements of technology. The substantially simpler methods of Myrbäck and Myrbäck (1931, 1932), and Bishop (1928, 1929, 1929a) are to be preferred even though they may still appear too complicated for the control laboratory. In general, it is sufficient to determine the simple fractions, permanently

soluble and coagulable nitrogen and formol titration in order to judge the proteolysis, and they have recently been used by the European research laboratories in increasing numbers. Loibl (1923) and more recently Lüers and Schild have analytically determined the changes in these nitrogen constituents during the malting process. The results are summarized in Figure 6.

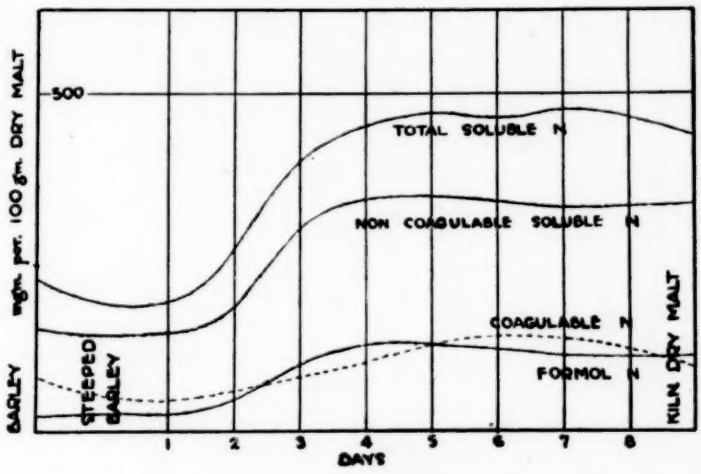

Fig. 6.

An impression of the total amount of proteolysis, conditioned principally by protein degradation, is obtained from the increase in soluble nitrogen, which as is known is quite considerable. For this purpose the variations in coagulable nitrogen are of much less significance. The formol titration increases greatly as a result of the more extensive breakdown by peptidases. That these large increases are actually accompanied by an increase in the concentration of the corresponding enzyme is shown by a study of Loibl (1923) who determined the formol titration before and after the destruction of the enzyme. As Figure 7 shows there is a large increase in the enzyme.

The nitrogen determined by formol titration serves as a measure of the low molecular weight, highly degraded nitrogen compounds of the malt, but we must not conclude that they are represented only by free amino acids since as is known the amino nitrogen of di- and polypeptides is also partially titratable. In order to obtain an estimate of how large the increase in the free amino acids is, Schild, upon the suggestion of Lüers, quantitatively determined by means of a new colorimetric method the amino acids cystine, tyrosine and tryptophane in the cold water ex-

tract of the malt. As Figure 8 shows, the increase in concentration of these free acids is not significant, but is quite different from and remains much less than that indicated by the formol titration.

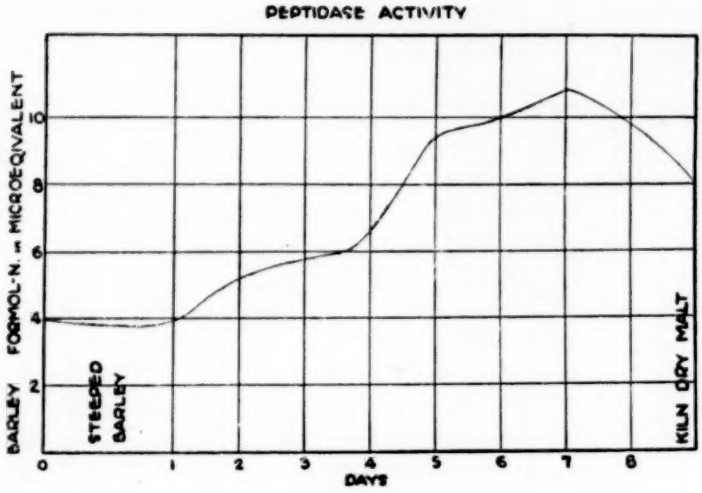

Fig. 7.

As with the carbohydrases, the drying process also has a destructive effect on the proteases which is much more pronounced because of the greater sensitivity toward heat of these latter enzymes. Aside from this the drying produces in the nitrogen compounds considerable altera-

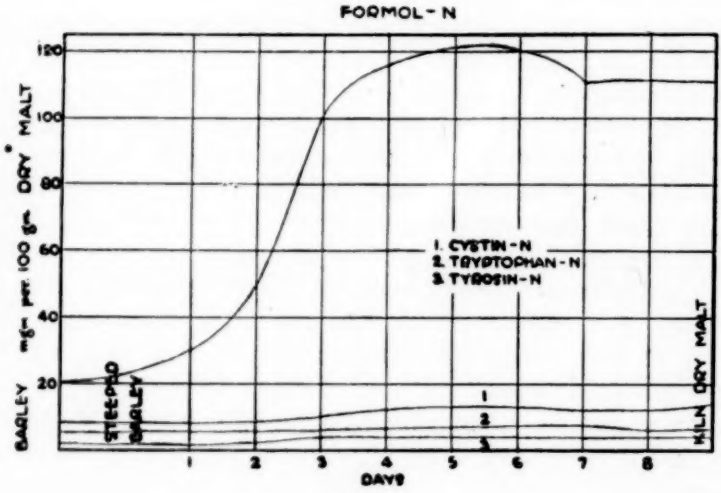

Fig. 8.

tion in consequence of chemical reactions such as coagulation of proteins, combination of amino nitrogen and sugars to form melanins according to Maillard (1912, 1912a) and Lintner (1912). This subject, however, lies outside of our considerations.

We also observe that the amount of proteases in malt depends upon the length of germination and the temperature. A cool malting gives a higher proteolytic activity in the malt than a warm one (see Lüers *et al.*, 1934). Moreover, peculiarities of the crop year produce a marked effect as the technologist well knows. "Heating" of barley is a phenomenon which for the most part is connected with the kind of nitrogen compounds in the grain and the enzymic transformation during sprouting, as Delbrück (1906) and Reichard (1919) have shown.

In concluding this discussion of proteases it might be mentioned that, as indicated above, according to the results of Lüers (1929), Kolbach (1932, 1933), Bishop (1929a), the analytical estimation of a few easily determinable nitrogen fractions gives the technologist a means of judging the correct course of proteolysis. Without going into detail, the following figures for an easily soluble, brilliant malt can be designated as normal:

Soluble N in % of total N determined in cold water extract	30.5 to 33.5%
Coagulable N in % of total N determined in cold water extract	7.5 to 8.5%
Permanently soluble N in % of total N determined in cold water extract	22.5 to 26.0%
Formol N in % of total N determined in wort	10.5 to 12.5%

Esterases

Along with carbohydrases and proteases the esterases also demand our attention since they give rise to inorganic compounds, particularly phosphoric acid, which serve as buffers and yeast foods. The characteristic esterases of the lipase type which split the true fats are undoubtedly important for the nutritional processes which include the translocation and metabolism of the fats. Little is known about these enzymes in the germinating grain (see vonLaer, 1921a, and Maestrini 1921), the investigations having been confined to the qualitative and quantitative changes which the fat undergoes from barley to malt (see also Sedlmeyer 1921, and Täufel and Rusch 1929, 1929a).

Phosphatases

The phosphateses are well known principally from the work of Lüers and Malsch (1929) and Lüers and Silbereisen (1927). As Figure 9 shows, the phytase, glycerophosphatase, nucleotidase, and saccharophosphatase studied by these authors are considerably increased in concen-

tration during malting as they all lead to the production of inorganic phosphates from organic phosphoric acid esters.

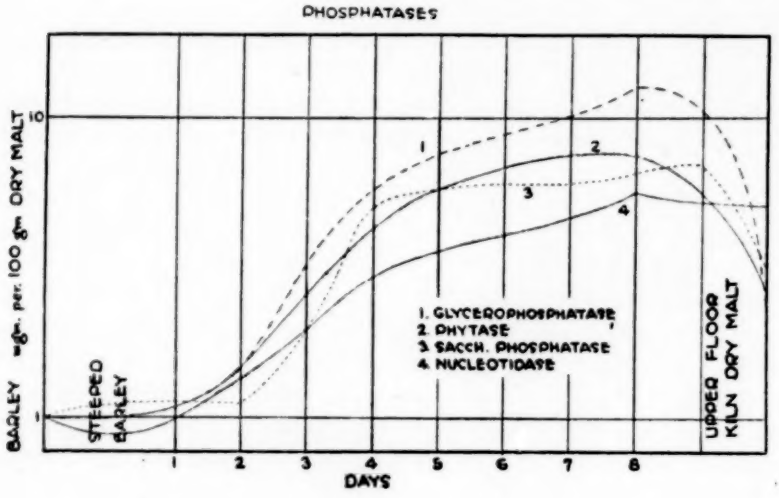

Fig. 9.

Their very great sensitivity toward high temperatures is worthy of note, they are therefore partially destroyed during drying. It can also be shown that a cool, slow malting produces a malt with the largest enzyme content. Lüers, von Zychlinski, and Bengtsson (1931) also isolated from malt the enzyme pyrophosphatase, characterized by its ability to hydrolyze pyrophosphates, which are important in the biochemistry of cell metabolism, to orthophosphate. The phosphates are vitally concerned with the development of acid which is observed during malting, which Lüers and Adler (1915) and Loibl (1923) have shown is likewise enzymic in nature.

Desmolases

The enzymes considered previously are all characterized by their ability to degrade more or less complicated organic compounds into simple units through the course of hydrolysis. On the other hand, there is another group of enzymes which are concerned with the energy transformations of respiration and fermentation. They usually attack the simple carbohydrates and break the long chains into simpler units through cission of the carbon-carbon bond, they are thus concerned with oxidation-reduction enzymic transformations.

It is customary to designate this group of enzymes as desmolases.

Only few enzymes of this type have been studied in germinating barley. Gracanin (1927), Bach and Oparin (1922), Loibl (1923), Charmandarjan (1930), Myrbäck and Myrbäck (1932a) studied the development of catalyse, the hydrogen peroxide splitting enzyme, and found, as Figure 10 (taken from Loibl, 1923) shows, a three or four fold increase. Peroxidase, however, remains substantially unchanged according to Loibl (1923), while Bach and Oparin (1922, 1922a) found a significant increase for peroxidase and oxygenase.

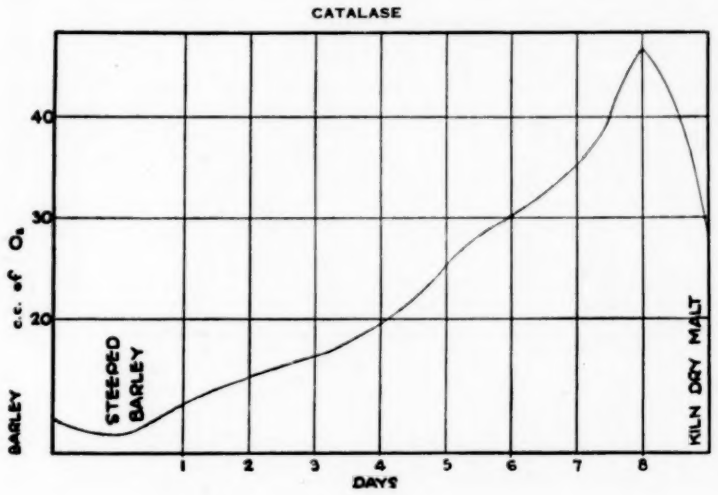

Fig. 10.

Finally, the energy transforming enzymes are manifested most obviously in the very easily determinable respiration of the germinating grain. Among many individual observations of respiration (see Hesse, 1920, 1934), an interesting old study of Abrahamsohn (1910) may be mentioned. The most important result of this work is that a barley with high protein content and low thousand corn weight, that is a small grained barley, respires most. As a consequence higher protein content always leads to a more active respiration than low protein content. Recently Kühles (1929) at our suggestion has measured with modern methods the intensity of respiration of the germination grain and found the results illustrated in Figure 11.

The large increase of respiration with accelerating growth and the diminution resulting from the drying process is obvious. Kühles also found that increase in temperature of germination produced a very considerable increase in rate of respiration. Since the respiration accounts for a considerable portion of the malt shrinkage, the effort of the tech-

nologist to minimize respiration as much as possible by means of cool malting or by minimizing the production of carbondioxide, is understandable. This can only go so far, however, so that the vitality of the sprout is not endangered and with it the uniform and automatically

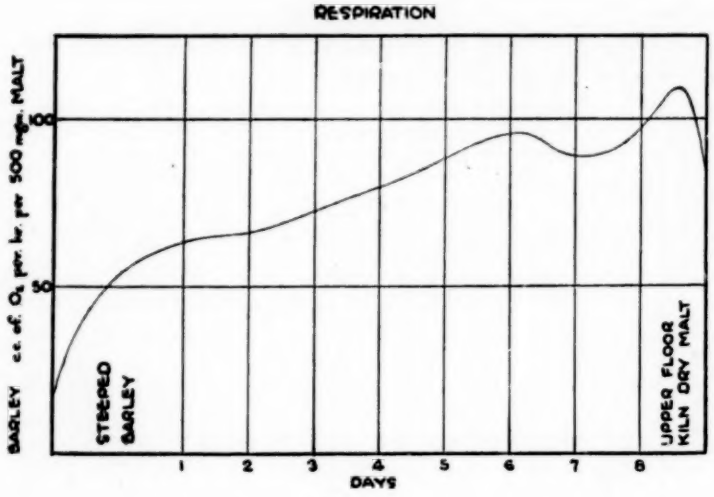

Fig. 11.

regulated metabolic activity, while at the same time the enzyme concentration necessary to the transformation of the contents of the grain and other chemical action is actually attained, which of course is the principal aim of the maltster.

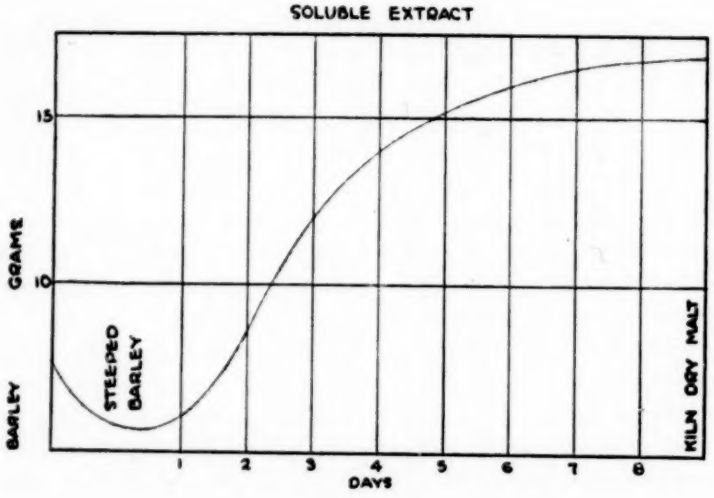

Fig. 12.

Considering now in total the enzyme processes in germinating grain we find them all manifested in some degree in the amount of cold water soluble extract at the time under consideration. It must be remembered, however, that the results appear to be low because of the respiration loss. In Figure 12, the change in cold water extract is graphically presented as taken from a study of Lüers and Schild. From a study of Figure 12 the remarkable increase accompanying the progressive germination and solution process is apparent.

In the exposition of this material the shortness of the time at my disposal has frequently imposed regretable limitations; the subject could be covered only in its bolder aspects. Nevertheless I still trust that I have been fortunate enough to have presented a glimpse of the wonderful, manifold and masterly methods of nature, a glimpse which inspires not only our highest reverence but also our humble admiration for the marvel that is life.

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SELENIUM IN PROTEINS FROM TOXIC FOODSTUFFS.[1] II. THE EFFECT OF ACID HYDROLYSIS

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This is the second paper of a series of investigations designed to determine the role and chemical nature of selenium (Robinson, 1933) in the toxic foodstuffs reported by Franke (1934, 1934a). This work was begun to find methods of removing the selenium from toxic proteins, and to determine if the removal of selenium will result in a non-toxic (normal) product. The isolation and identification of the toxicant is the ultimate goal. It has been found (Franke and Painter, 1935) that the selenium is bound in the protein molecule in such a manner that it is in solution only after the protein has been hydrolized. It will be shown that these products of acid hydrolysis are toxic.

Part of the selenium in toxic protein hydrolysates has been found in the humin (Franke and Painter, 1935) formed when these proteins are hydrolized with acids.

Experimental

The method used for detecting the selenium was previously described (Franke and Painter, 1935). Conclusions will be drawn only when the difference in selenium content determined by this method is convincing. The relative amounts of selenium were based on color developed from equal weights of the sample tested and not on the total weight of the fractions. These were compared with the original protein. In some cases when material was available, the selenium was determined by the method described by Robinson *et al.* (1934). The source and method of separation of the protein used in this investigation has been mentioned in a previous paper (Franke and Painter, 1935). The nitrogen content of this protein was only 13.3%, although it was dispersed in 0.075 N acetic acid and precipitated by the addition of sodium chloride. Following the procedure of Robinson *et al.* (1934) this protein was found to contain approximately 100 p.p.m. of selenium. Hydrolyses of proteins were carried out in round bottom flasks fitted to water

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condensers with ground glass joints. The digestions were carried out on sand baths. The humin was filtered off, and washed several times with hot water.

Hydrolysis with 20% hydrochloric acid: When toxic proteins were hydrolized by boiling with 20% hydrochloric acid for 20 hours, the selenium content in the humin (mgr. percent) formed appeared somewhat greater than that of the original protein. However, the quantity of humin formed was not great enough to greatly reduce the selenium content in the hydrolysate.

Hydrolysis with sulfuric acid: Most of the toxic proteins have been hydrolized by boiling for 14 hours in three times their weight of concentrated sulfuric acid and six times their weight of water (Mitchell and Hamilton, 1929). Sulfuric acid was preferred because it could be readily removed with barium, and the hydrolysate used in feeding experiments.

The weight of the humins, and also its selenium content, varied, although hydrolyses were carried out under identical conditions in every case. That the amount of humin formed varied has also been reported by Burr and Gortner (1924). The average weight of humin formed per 120 g. of protein by the method described was 8.3 g. This was based on the total humin from 5700 g. of protein. It was evident that the selenium content of the hydrolysate was slightly reduced (about $\frac{1}{8}$ of the selenium was removed).

When soluble selenium salts are added to selenium-free protein and this is hydrolized, the selenium is reduced to the metallic state and appears in the humin from which it can be removed with bromine in hydrobromic acid.

Since selenium appeared in the humin, the humin formation was increased to determine if the selenium content remained constant or decreased.

Many workers (Gortner and Blish, 1915; Gortner and Holm, 1917; Roxas, 1916; Gortner and Holm, 1920; Nollau, 1915; Grindley and Slater, 1915; Hart and Sure, 1916; Holm and Gortner, 1920) have shown that the addition of carbohydrates during acid hydrolysis greatly increases the amount of humin formed.

When dextrose was added to increase the amount of humin formed, the total selenium in the humin increased proportionally. From 20 to 24 g. of humin were formed when 40 g. of dextrose were hydrolized with 100 g. of protein. This humin contained approximately the same amount of selenium per gram as the humin formed when no dextrose was added. The selenium content of the hydrolysate was reduced to about one-half that of the protein. When 100 g. of dextrose were hydrolized with 100 g. of protein, the humin formed weighed 74 g.

The selenium content per gram of humin was reduced somewhat from that when the protein was hydrolized alone. The hydrolysate from this hydrolysis contained less than one-fourth the average amount of selenium for a "normal" toxic hydrolysate.

The weight of the humin was increased by hydrolizing with 50% sulfuric acid (Butz and du Vigneaud, 1932). The selenium content per gram of humin was not reduced. The hydrolysate contained about one-fourth the average selenium content.

By combining the addition of dextrose with 50% sulfuric acid, results similar to those above were obtained. In no case was all the selenium removed from the hydrolysate, but the content was perceptibly reduced. No experiments have been conducted using different carbohydrates, although some are more effective than others in increasing humin formation (Roxas, 1916, and Gortner and Holm, 1920). In many cases the selenium in the humin plus the selenium in the hydrolysate did not equal that in the protein hydrolyzed. Some selenium was lost in the barium sulfate when the sulfuric acid was removed from the hydrolysate. The humin resulting from the hydrolysis when 50% sulfuric acid was used has a gummy consistency and part of it could not be filtered out. This was removed with the barium sulfate.

Since the humin formed when toxic proteins are hydrolized with hydrochloric acid or sulfuric acid contains selenium in amounts sometimes greater than in the original protein, a consideration of humin formation may throw some light on the nature of selenium in toxic proteins.

The work of Gortner and his collaborators (Gortner and Blish, 1915; Gortner and Holm, 1917; Gortner, 1916) and of Roxas (1916) indicates quite conclusively that of the known amino acids tryptophane and tyrosine are concerned in humin formation. Tryptophane condenses in some way with an aldehyde group to form insoluble humin. The amino acid nitrogen from other amino acids in the humin formed is probably due to adsorption on the amorphous humin (Roxas, 1916, and Gortner and Holm, 1920).

The role of cystine in humin formation has not been elucidated. Dowell and Menaul (1919) state that there is strong evidence that cystine reacts. Lugg (1933) pointed out that the loss of cystine was due to adsorption and was not large when humin formation was small, while in the case of cysteine there is actual condensation with ketones and aldehydes forming mercaptols and mercaptals, respectively. He reported that when hydrolysis was carried out in the presence of stannous chloride the loss of cystine was enormous.

Hydrolysis in presence of reducing substances: Stannous chloride and

tin did not perceptibly change the selenium content in the humin formed when these reducing substances were added during hydrolysis. The amount of humin formed was not reduced.

Results with hydriodic acid hydrolysis: Since Baernstein (1933) has stated that no humin is formed when hydriodic acid is used to hydrolize proteins, thirty grams of toxic protein were hydrolized by boiling for 12 hours with 200 cc. of 57% hydriodic acid. After hydrolysis, concentrated sulfuric acid was added to oxidize the hydriodic acid. The free iodine was extracted with carbon tetrachloride. The iodine left in the solution as hydriodic acid was extracted with ether. This left a colorless hydrolysate. A few cubic centimeters of dilute silver sulfate solution were added to determine if any hydriodic acid remained. After a few minutes a faint precipitate formed. This was filtered off. The sulfuric acid was removed by barium hydroxide and the amino acids dried. These were selenium free.

Isolation of tryptophane: Since tryptophane condenses with certain substances to form insoluble humin (Gortner and Blish, 1915; Gortner and Holm, 1917; Gortner, 1916; and Roxas, 1916), Miss Marx (unpublished data) attempted to isolate tryptophane from toxic proteins hydrolized by barium hydroxide according to the method described by Waterman (1923). When the mercury-tryptophane was decomposed with hydrogen sulfide, some selenium was found in the free tryptophane, but much more was in the mercuric sulfide. The filtrate from the mercury-tryptophane precipitate still contained selenium.

Isolation of tyrosine: Tyrosine, the other amino acid known to contribute to humin formation, was crystallized from a toxic protein hydrolysate by concentration. This preparation contained a trace of selenium, but this was probably present as a contaminant.

Discussion

Since selenium is in combination in the protein molecule of toxic proteins in such a manner that it is liberated in some organic compound or compounds as one of the products of protein hydrolysis, it appears probable that it may be in some unidentified amino acid.

Since selenium appeared in the insoluble humin formed when toxic proteins were hydrolized, it appeared probable that it was in a compound in many respects similar to tryptophane. This is the only amino acid condensing in some manner to form insoluble humin (Gortner and Blish, 1915; Gortner, 1916; and Gortner and Holm, 1917). When this amino acid was isolated from a toxic protein hydrolysate, the selenium content of this preparation was small. This may have been due to the decomposition of the selenium compounds during alkaline hydrolysis. The selenium content of toxic proteins is inde-

pendent of the tryptophane content because zein, a protein very low in tryptophane, from toxic corn contains selenium in amounts comparable to other toxic proteins.

Tyrosine, the other amino acid known to contribute part of its nitrogen to humin formation (Gortner, 1916; Gortner and Holm, 1917; and Roxas, 1916), was contaminated with only a small amount of selenium when it was isolated.

By close examination of the methods used in isolating these two amino acids, it is evident that in both cases a small amount of cystine would have been present. Hopkins and Cole (1901) used their reagent to precipitate cystine from the products of hydrolysis. Histidine would also have been precipitated. The work of Gortner and Sinclair (1929) indicates that hydrolysis with barium hydroxide would not quantitatively decompose cystine as Waterman (1923) stated. The solubility of cystine is near that of tyrosine so it is practically impossible to crystallize tyrosine from a hydrolysate without obtaining some cystine. From this it appears as though one selenium-containing compound had properties very similar to those of cystine.

In its chemical properties selenium resembles sulfur very closely. It is not entirely improbable that selenium has in part replaced sulfur in the two known sulfur containing protein hydrolysis products, cystine and methionine. Bradt and co-workers (1929, 1929a, 1930, 1931, 1934) have prepared an exhaustive review of organic selenium compounds. Examination of this review reveals that for nearly every type of organic sulfur compound a selenium homologue has been prepared, and that these selenium compounds resemble those of sulfur very closely in both preparation and reaction.

Byers (1934) found selenium, arsenic, chromium, and vanadium in a soil sample upon which toxic grains were grown. Selenium has been found in nearly all soils in the area producing toxic foodstuffs. It is possible that selenium may be taken up and assimilated by plants in a manner analogous to sulfur. Hurd-Karrer (1933, 1934) has shown that the addition of sulfur to selenium-containing soils will prevent the absorption and assimilation of selenium by plants.

Based on 120 p.p.m. selenium in the protein used, the molar selenium-sulfur ratio is 1 : 148. The sulfur content was determined by the method described by Frear (1930). If selenium is in some compound (or compounds) similar to those of sulfur, the difficulty in separating the two is obvious.

Whether or not the selenium compound (or compounds) is selectively adsorbed on the humin or condenses to form humin substances cannot be stated.

Lugg (1933) has stated "that the simple aliphatic mercaptans

readily condense with aldehydes and ketones forming mercaptals and mercaptols, respectively." His work would suggest that if a selenium homologue of cystine or homocystine were present and reacted similarly to them, the selenium content of the humin formed should be greatly increased when stannous chloride was present during the hydrolysis.

No explanation is given for the fact that hydriodic acid hydrolysis gave a selenium free hydrolysate. A selenium iodide may have been formed, but by adding bromine in hydrobromic acid to a sulfuric acid hydrolysate of toxic protein and reducing with sulfur dioxide and hydroxylamine hydrochloride (Robinson, 1933) the selenium present is not reduced to the metallic state as in the case of inorganic selenium salts. Baernstein (1933) uses concentrated hydriodic acid in his method for determining methionine because it demethylates the $-S-CH_3$ group. Part of the selenium was recovered in the iodine extracted by the carbon tetrachloride.

Summary

When hydrochloric acid and sulfuric acid are used to hydrolize toxic proteins, a part of the selenium appears in the insoluble humin and a part in the soluble hydrolysate. By increasing humin formation by the addition of carbohydrates or hydrolizing with stronger acids, the proportion of selenium in the humin fraction can be greatly increased.

Hydrolysis with hydriodic acid resulted in a selenium-free hydrolysate.

Isolated tryptophane and tyrosine contained only small amounts of selenium, which probably was a contaminant.

The possibility of selenium replacing the sulfur in the amino acids cystine and methionine appears probable.

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PHYSICAL TESTS TO DETERMINE QUALITY IN WHEAT VARIETIES[1]

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Standards for Quality in Wheat

In testing wheat varieties for quality it is necessary to have standards by which the values of new and unknown varieties may be judged. Since wheats and flours are used for a variety of purposes and under many different conditions, the question arises, where shall the standards be found and what considerations shall govern in the adoption of these standards? Successful milling as well as baking was accomplished before the establishment of wheat and flour testing laboratories. Millers learned from experience that wheats of certain appearances and which had grown in certain regions were better adapted for particular purposes than other wheats of different appearances and grown in other regions. The standards and methods used in testing wheats for quality thus have their origins in practices which have existed longer than the modern laboratories. In this evolutionary process certain wheats have become known as having qualities desired for particular purposes. The hard red spring wheats were among the first in the United States to establish distinct standards for bread wheats. These were soon followed by the Turkey group of hard red winter wheats. These two groups of wheats or the varieties within these groups may then be considered to have set the standards for testing new varieties of hard wheats which are to be used in bread making.

Soft red winter wheat flours and some white wheat flours have been shown to have qualities desired for baking when sodium bi-carbonate is the leavening agent. Where the qualities inherent in typical soft wheat flours are desired, characteristics associated with hard wheat flours are as objectionable as the soft wheat flour characteristics are in hard wheat flours. Therefore, in breeding or developing soft wheats the standards

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of quality should be entirely different from those used for evaluating the hard wheats, intended mainly for bread making. At the present time standards for soft wheats are less definite than are those for the hard bread wheats. Since soft wheats are used for a large variety of purposes, and since there are many more varieties of soft wheats, it is more difficult to select any one variety or group of varieties as a standard. It is probably better to select as standards soft wheat flours which are used for specific purposes and evaluate the new soft wheats and their flours in comparison with these flours.

Wheat is valued as a bread grain because of the peculiar properties of its gluten. Bread is universally liked as a food, not only because of its content of energy and protein material, but because it has these in a peculiar physical form. Bread has a porous structure of very thin cell walls presenting an enormous surface to the action of the organs of taste and to the digestive juices. Here then is the core of the quality problem in bread wheats. The desirable quality is measured by the extent that the flour from the wheat will make a dough which when properly fermented by yeast and then set by the heat in the baking oven will make a porous structure of thin cell walls of an enormous surface. There are other quality factors, but if this one is lacking, the others are of no avail. The spring wheats, particularly Marquis, and the winter wheats, especially Turkey, have shown themselves to possess gluten which meets this demand of quality and hence they serve as satisfactory standards.

Gluten Determines Dough Structure

Gluten is the most characteristic ingredient in dough and without gluten dough would not form. The two most important properties of dough are plasticity and elasticity (taking form when pressure is applied and recovering form when pressure is removed). Elasticity is due mostly to the properties inherent in the gluten. These two properties make possible the expansion of dough and hence the production of bread. Quantitatively the amount of gluten in bread dough is relatively small. In a dough made from a 11% protein flour, the amount of gluten material is somewhat less than 7%, while the starch and water constitute about 46% each. The properties of the starch are important, but they have received less attention than they deserve. The gluten of dough apparently exists as a very fine attenuated network in which the starch granules are enmeshed. The water is adsorbed on the starch granules, on the gluten strands, and a small part exists as free water in the gluten mesh and among the starch granules. The amount of water in the free condition is of most importance in contributing to the dough its elastic and plastic properties. This makes it highly important to use the correct amount of water in dough.

Main Factors Influencing Cell Formation in Dough

The three main factors involved in the formation of the cell wall structure in dough by yeast growth are: (1) favorable conditions for the functioning of the yeast cells, particularly yeast food, (2) expansibility, which allows for the expansion due to the production of carbon dioxide, and (3) the retention of this gas by the cell walls. Weakness of the latter would produce large cells but with thick walls because of coalescence, a structure less desirable in bread than small cells with thin walls. Therefore, a fine, soft, even, silky texture is considered as very important in judging the quality desired in a bread wheat.

The behavior of dough under expansion due to yeast growth is different from that due to soda. Under the action of soda the expansion is comparatively sudden and then ceases. Under yeast action the expansion is gradual and ever increasing until the cells burst because of an ever increasing thinness. The extent to which these cells expand without bursting is an important quality characteristic. This is the reason that loaf volume together with texture has acquired an important place in measuring the quality in bread wheats.

Dough Formation

When about 3 parts of water are added to 5 parts of flour there is very little diffusion of water into the flour unless there is mechanical agitation. The dough films formed at the interface between flour and water inhibit deep penetration of water into the dry flour. Mechanical agitation breaks up these interfacial areas into smaller and smaller dimensions, thus giving chance for the formation of new contacts or interfaces between flour and water. When all the flour particles have been wetted, the water exists in all degrees of absorption, from that so firmly absorbed as to partake of the nature of bound water, to that which has the characteristics of a film in which the molecules have freedom of motion approaching that in a liquid surface. The amount of freedom of the water molecules in these interfacial surfaces is of the greatest importance in giving to the dough its plastic and elastic characteristics. If there is too much freedom, the dough is too slack, and if there is too little freedom the dough is too stiff. For this reason, obtaining the correct absorption is of the greatest importance in testing flour for its bread making quality.

Condition of Gluten in Dough

The mechanical manipulation with the fingers, necessary in gluten washing, squeezes out the starch from the gluten network and assembles the gluten into a wad. Some starch always remains, probably because

many of the finer starch granules swell and become so entangled in the gluten mesh that removal is impossible. Other substances such as fiber, fat, and ash also remain in the mass.

When dough is severely mixed it becomes more and more difficult to separate the starch from the gluten as the amount of mixing is increased, and after a while it is impossible to wash the gluten from the dough by the usual process. It is not exactly clear just what happens. It seems that the fibrils either break by the continuous stretching or they become very attenuated. In the latter condition, they would adhere so strongly to the surface of the starch granules that separation is very difficult. This adherence may be due to adsorption of solid on solid, or adherence of solids to each other by means of adsorbed liquids. That there is a large difference in the size of the starch granules and the gluten strands is apparent from the fact that starch granules can be easily seen under the ordinary microscope, while the gluten strands have a dimension in the range of ultramicroscopic particles. Thus, intimate diffusion of the attenuated gluten fibrils among the starch granules appears to be one reason why gluten cannot be washed from overmixed dough.

If dough overmixed just enough to prevent gluten washing is allowed a rest period, then it becomes possible to wash out the gluten. The separated and attenuated gluten filaments apparently come together forming larger strands. The gluten filaments have a greater amount of water adsorbed on their surfaces in proportion to their mass than the starch, making them flow together. When this happens the many filaments enclosing the starch unite into fewer strands. This condition would be more favorable for the removal of the starch from the gluten meshes. That is, after the dough has rested, it goes back to some extent to the condition which existed before the overmixing took place. Mixing may, however, be carried so far that recovery does not take place.

Mixing versus Fermentation Tolerance

Just what relationship exists between resistance to extended mixing and fermentation tolerance is not so clear. One difficulty lies in the lack of a common understanding of the meaning of fermentation tolerance. Some users of the Brabender Farinograph make these statements: "The length of time during which the maximum elasticity exists along the horizontal 500 line is an indication of the fermentation or mixing tolerance of the flour," and "the rate at which the curve declines indicates the rate of deterioration of the gluten due to mechanical action or to fermentation." These statements imply that these users of the Farinograph believe that there is a close relationship between fermentation tolerance and behavior towards severe mechanical

action. That severely mixed dough can be baked into bread having a good texture has been shown by Swanson and Working (1926). While fermentation involves mechanical action in the stretching and restretching after punching the dough, the by-products of fermentation, notably alcohol and acids, also have an effect on the gluten. These substances can act on the gluten material either because of their effect on the gluten proteins, notably gliadin, or because they influence the surface tension of the water films which help bind the gluten material together.

Fermentation tolerance has sometimes been confused with the phenomena due to lack of sugar or diastatic activity. Manifestly, if large amounts of yeast are used in baking so as to deplete the sugar rapidly, or if the fermentation period is prolonged so as to use all the sugar, a poor loaf will result no matter how good or strong the gluten. Any such meaning of fermentation tolerance leads to more confusion.

Mechanical Devices for Testing Gluten Quality

When dough expands under yeast action, the gluten network is subjected to a certain amount of mechanical stress, and the behavior against this stress when properly measured may be a test of its quality. Various devices have been used for measuring and recording the behavior of gluten in the dough under mechanical stress. A few of the typical devices will be mentioned and then most consideration will be given to curves obtained on the recording dough mixer (Swanson and Working, 1933) in use at the Kansas Agricultural Experiment Station for testing wheat varieties.

The Chopin (1927) Extensimeter tests mainly the extensibility and elasticity of the dough. A piece of dough prepared in a dough mixer is made into a thin sheet. This sheet is expanded into a bubble under air pressure so controlled that both the force and amount of pressure are known. The pressure is continued until the bubble bursts. By means of a pressure gauge a record in the form of a curve is made of the variations in pressure throughout the test. Thus the apparatus tests the extent to which the dough sheet may be stretched and also other tensile strength factors. The aim is to imitate the stretching of the dough cells under the influence of yeast action. This apparatus is used not only by the largest milling company in France, Grand Moulins de Paris, but is also used by the Vilmorin-Andrieux wheat improvement station near Paris. One advantage of this apparatus is the much smaller amount of wheat needed as compared with the experimental milling and/or baking tests. The Chopin Extensimeter, however, does not seem to have gained wide favor, which may be because its successful operation requires a high degree of skill.

The Bühler Comparator [2] also tests the extensibility of a sheet of dough. Instead of using air pressure the expansion is made with a rounded wooden cone. The dough sheet is held in place while the cone is pushed up and the dough is stretched over the cone in a sheet of gradually increasing thinness. The extent to which the sheet can be stretched before it breaks is a measure of the extensibility and hence the strength of the gluten. The behavior of the dough under this stress is also recorded in a curve. This instrument has a wide use in the mills in Europe, but the writer does not know of any instances where it is used in wheat improvement work.

The Brabender Farinograph (Brabender 1932, 34) is designed to measure the " developing time " which depends on the speed with which the dough absorbs water, the consistency and elasticity of the dough, and the length of time this elasticity resists mechanical action. The Farinograph has certain features in common with the Swanson and Working Recording Dough Mixer (Swanson and Working, 1933), and there are also important differences. The main difference is that in the Farinograph there is more kneading and less stretching, and in the Kansas Recording Dough Mixer there is more stretching and less kneading. The treatment the dough receives in the dough mixer is much more severe than in the Farinograph, with the consequence of a more complete breakdown of the dough. This is shown in the more sudden and complete disappearance of the amplitude in the curves obtained from certain varieties. In the use of the Farinograph it is recommended that all doughs be mixed to a standard consistency indicated by 500 on the chart. This makes it possible to use this apparatus also for the determination of absorption. With the Recording Dough Mixer it is recommended that the absorption be determined by the method employing the supercentrifuge (Swanson and Working, 1933).

Operation of the Recording Dough Mixer

The mechanism which controls the recording pen is so adjusted that the pen will not move from the base line of the graph paper until the water and flour begin to unite and form dough, which requires about 10 seconds. At this point the resistance increases and the recording pen moves away from the base line. The graph paper moves with reference to the pen at such a rate that the time between the curved cross lines is exactly one minute. Hence the time it takes for the pen to move any distance from the base line or to reach the top of the curve is measured very accurately. Thus it is possible to estimate from the dough curve the time it takes the dough to reach any stage of development. The time for subsequent changes to take place is also accurately measured.

[2] Made by Bühler Brothers, Utzwil, Switzerland.

The ascending part of the dough curve is very irregular or jagged due to more or less developed chunks of dough. The regularity or irregularity of this part of the curve seems to have very little significance. The time the pen remains at the top, or the extent of rounding of the dough curve, indicates the duration of resistance against continued mechanical action. The rate of descent or dip of the curve, and the time until the dough begins to break or disintegrate, shown by the gradual disappearance of the amplitude, indicates sensitiveness to severe mechanical action. With some flours the breakdown is so rapid and so complete that the resistance decreases to such an extent that the dough curve becomes a straight line. With some flours breaking down is not complete and the pen moves back and forth until the end of the mixing time. The amplitude of this to and fro movement, particularly after the dip starts, as well as the character of the top, are important quality characteristics which are related to inheritance or variety.

Dough Curves from Selected Flours

In Figure 1 are shown six dough curves from as many selected flours. These flours were obtained from mills and bakeries. The spring wheat flour was obtained from a mill so situated that it would use " strong " spring wheat. Other dough curves made from time to time on flours from this mill show very similar characteristics and hence this curve may be taken as a type of the " strong " bread flours made from spring wheats. The Kansas hard wheat flour was obtained from a bakery in Kansas City and this flour was rated good for sponge dough bread. The Wichita flour was also obtained from a bakery and was reported to give good results. The name " Wichita " indicates that the flour came from a mill in that territory and was probably milled mostly, but not altogether, from Blackhull wheat, since this wheat is widely grown in the Wichita area. The cracker flour was obtained from a mill located in Missouri and was manufactured for the cracker trade. The cooky flour was obtained from a bakery in Chicago and was reported to be made from club wheat. It was an unbleached flour. The cake flour was obtained from a bakery in Kansas City and was used for general cake baking.

These dough curves may then be taken as illustrating the types of dough behavior that will be obtained from flours used for specific purposes.

Meaning of Dough Curve Characteristics

The low protein soft wheat flours, like the cooky and cake flours, have a low mixing tolerance as shown by the smaller area under the curve and the rapid breaking down resulting in smooth lines. The

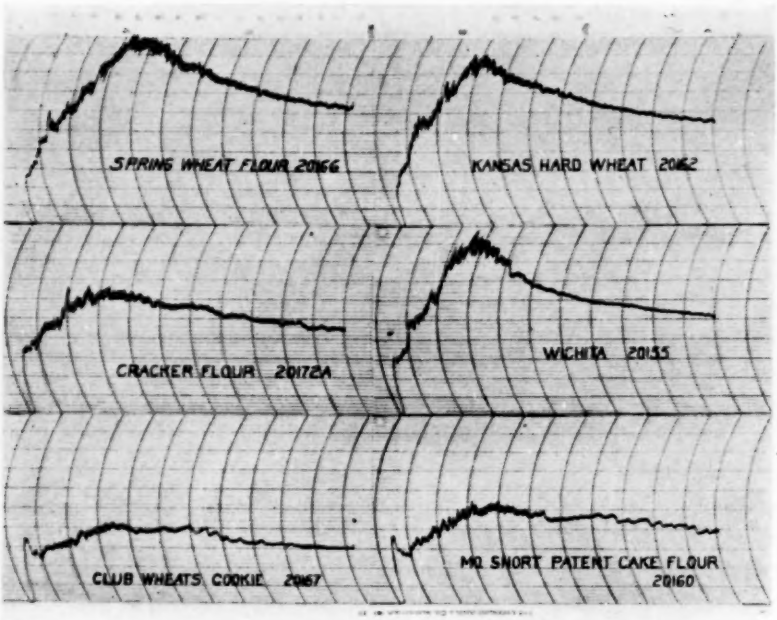

Fig. 1. Curves of flour obtained from mills and bakeries. The flours were as follows:

Spring wheat flour, Minneapolis Mill.
Cracker flour, Kansas City mill.
Cookie flour from Club wheat, Chicago Bakery.
Kansas Hard wheat, Kansas City Bakery.
Flour, Michigan Bakery.
Missouri Short Patent cake flour, Kansas City Bakery.

The curves from the spring wheat, Kansas hard wheat, and the flour milled in Wichita, Kansas, may be taken as typical of those which should be obtained from flours of wheats that should be satisfactory as bread wheats. The curves from the cracker, cookie, and cake flours represent the characteristics desired in soft wheat used for making flours for baking with soda as the leavening agent.

larger up and down movements in the curve after the amplitude disappears are due to a stickiness of the dough causing it to adhere to the center of the bottom of the bowl. A sudden dip indicates a loosening after which it began to stick again and increase the resistance, as shown by a rise in the curve. So far we have not found any special significance in this particular behavior.

The bread flours, like the spring wheat and the Kansas hard winter flours, have large areas under the curves, rounded tops, and the amplitude persists to the end. In the curve from the "Wichita" flour the amplitude almost disappears. This flour is probably largely from Blackhull wheat, but not altogether. As will be shown later, the curves of flours made from Blackhull wheats are fundamentally different from those made with the hard red spring and the hard red winter varieties like Turkey, Kharkov, and Kanred. When Blackhull wheat first came on the market there was much complaint about this variety. Some bakers said that the flour was lacking in mixing and fermentation tolerance. When these two factors were adjusted to suit the flour, the troubles began to disappear.

Use of Dough Curves in Testing Wheat Varieties

Obtaining dough curves on the recording dough mixer is now a regular part of the routine in testing wheat varieties at the Kansas Agricultural Experiment Station. While the characteristics of these dough curves are not taken as final indications of the quality of new and more or less unknown varieties, it has been found that varietal factors in wheat quality are more accurately revealed by these curves than by any other one measure which is available for testing quality. That is, if the dough curves obtained from new varieties are similar to those obtained from varieties now known and accepted by the trade there is very good reason to believe that further tests for quality will show that the varieties have satisfactory baking characteristics. Since quality is a relative term, and also since the quality of a variety must be judged in reference to its intended use, the characteristics of the dough curve as indications of quality must also be considered in the light of the intended place the new variety shall occupy among the commercial wheats. To illustrate: If the dough curve of a new variety is very similar in characteristics to the dough curve obtained from the Turkey or Marquis varieties, there is very good reason to believe that the new variety will be satisfactory as a bread wheat. On the other hand, if the dough curve of a supposedly hard wheat variety is similar in some respect to dough curves obtained from soft wheat varieties then it is very probable that the new hard wheat variety has certain weaknesses in quality and that these are inherited. Likewise, if the dough curve from a supposedly soft wheat variety has some of the characteristics which prevail in hard wheats it is very probable that this variety will not be suited to the strictly soft wheat trade.

Dough Curve Characteristics in Varieties Representing Wheat Classes

In the past few years dough curves have also been made from a large number of flours representing different commercial classes of wheats and varieties within these classes. From among these numerous dough curves several have been selected to represent the following classes and varieties of wheat:

1. Hard red spring wheats: Marquis, Ceres, Reward, and Thatcher.
2. Hard red winter wheats:
   - *a.* Turkey and Karkov.
   - *b.* Tenmarq.
   - *c.* Blackhull and Early Blackhull.
   - *d.* Cheyenne, Oro, and Cooperatorka.
   - *e.* Kanred and Quivira.
3. Soft wheats grown in other states.
4. Kansas soft wheats: Fulcaster, Kawvale (semi-hard), Harvest Queen, and Clarkan.

The hard red spring wheats (*Figure 2*). With the dough curves illustrating the flours milled from the well-known variety Marquis, are grouped some of the curves obtained from the flours milled from the newer, but accepted, spring wheat varieties (Ceres, Reward, and Thatcher). Tests made on flours milled from these varieties have shown them to be comparable in quality to Marquis flours, the latter being considered the standard of quality in hard red spring wheats. All

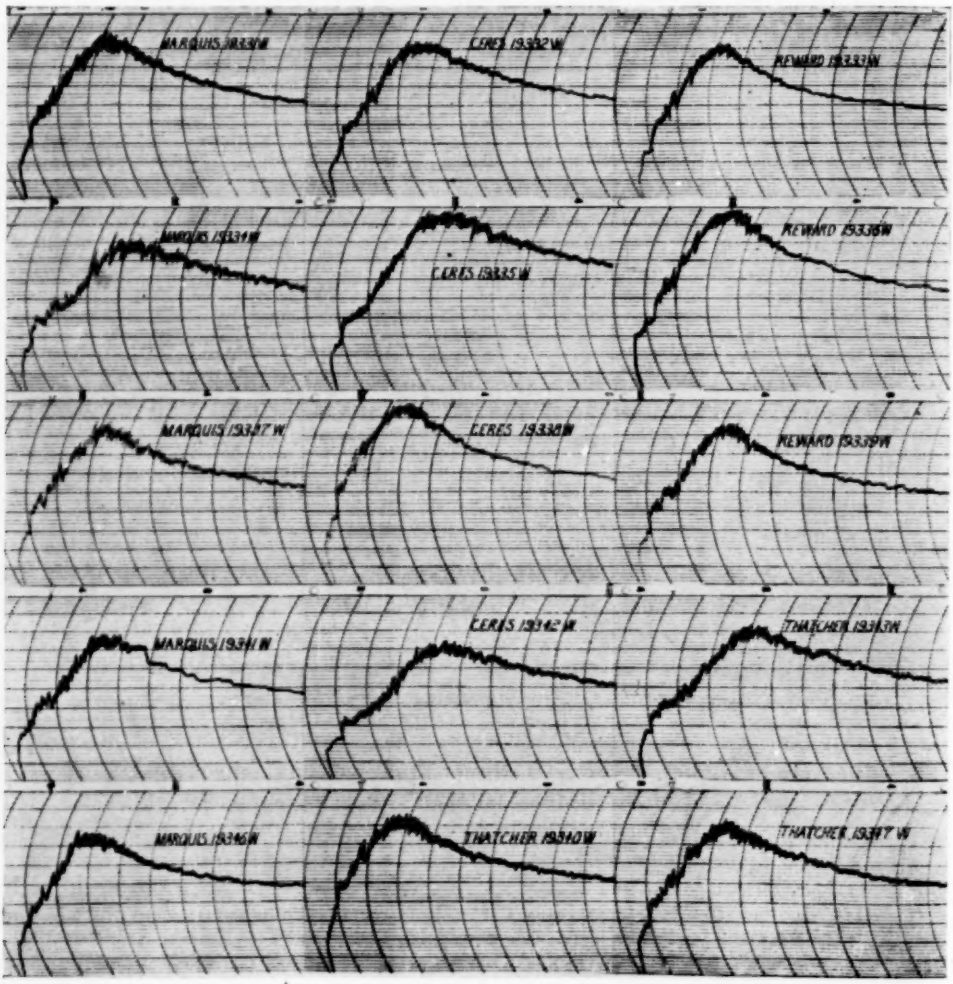

Fig. 2. Curves made from flours of spring wheats. The localities of the wheats, grown in 1933, were as follows:

Marquis, Fessenden, N. D.	Ceres, Fessenden, N. D.	Reward, Fessenden, N. D.
Marquis, Leeds, N. D.	Ceres, Leeds, N. D.	Reward, Leeds, N. D.
Marquis, Fargo, N. D.	Ceres, Fargo, N. D.	Reward, Fargo, N. D.
Marquis, Ft. Benton, Mont.	Ceres, Ft. Benton, Mont.	Thatcher, Ft. Benton, Mont.
Marquis, Crookston, Minn.	Thatcher, Fargo, N. D.	Thatcher, Crookston, Minn.

These may be taken as typical of the characteristics in curves expected of flours from strong spring wheats which have a high reputation as "bread wheats."

of the spring wheat flour curves show medium slow development, about 3 minutes, rounded top, gradually decreasing amplitude, but no complete breaking resulting into a smooth line. The protein content of most of the spring wheat samples was rather high. No close correlation, however, between the protein content of the wheat and the general character of the curves was found. The three curves representing the

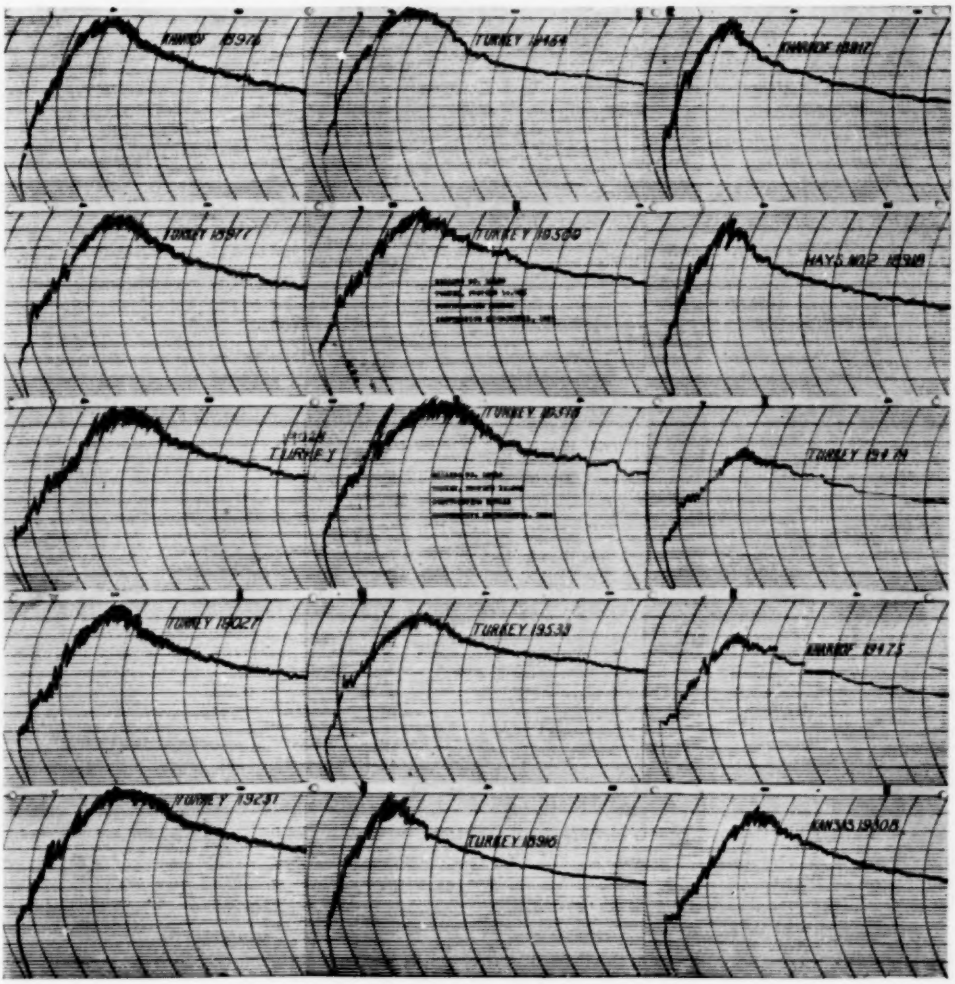

Fig. 3. Curves obtained from flours made from hard winter wheats. The localities of these wheats grown in 1933 and 1934, were as follows:

Kharkof, Hays, Kans.	Turkey, Hays, Kans.	Kharkof, Manhattan, Kans.
Turkey, Hays, Kans.	Turkey, Southwest, Kans.	Hays No. 2, Manhattan, Kans.
Turkey, Northwest, Kans.	Turkey, Northwest, Kans.	Turkey, Manhattan, Kans.
Turkey, Southwest, Kans.	Turkey, Manhattan, Kans.	Kharkof, Manhattan, Kans.
Turkey, Manhattan, Kans.	Turkey, Manhattan, Kans.	Hard wheat flour, Kansas mill.

These curves are very similar in characteristics to those obtained from spring wheats (Fig. 2), and indicate what should be obtained from new varieties of hard winter wheats.

variety Thatcher are remarkably similar, although the three samples were grown in three different states. The dough curves representing the variety Reward are also strikingly similar. This is also true of the curves for the variety Marquis. The greatest variation in curves was found with the flours milled from the variety Ceres; nevertheless they had the general characteristics of the other spring wheats.

The Turkey wheats (*Figure 3*). The general characteristics of the dough curves obtained from the Turkey wheat flours are strikingly similar to those obtained from the hard red spring wheat flours. In two of the dough curves (Turkey No. 19474 and Kharkov No. 19475, samples grown in field plots, Manhattan, 1934), the amplitude disappeared. These wheats, however, were of low protein content and grown in a generally high protein year. Turkey No. 18918 from the field plots grown at Manhattan in 1933, shows a more than usual rapid development. All in all, these dough curves may be taken as representative of strong hard red winter wheats. The last dough curve, illustrating Kansas No. 19308, was made on a flour from a mill located where it would obtain, as a rule, Turkey wheat. While the dough curves shown in Figure 3 do show considerable variation, it is not more than would be obtained in baking tests made on flours milled from wheats grown at Manhattan and Hays, Kansas, as well as in various parts of the state.

Very wide variations in protein content seem to have an influence on the contour of the curve. The protein contents of Kharkov No. 18976 and Turkey 18977 were 19.1% and 19.2%, respectively, and of Turkey No. 19474 and 19475, 11.4% and 12.6%, respectively. The curves illustrating the last two samples show weakness and the same weaknesses were revealed by baking tests. The amplitude, however, persists showing that the breakdown was not complete.

The Tenmarq samples (*Figure 4*). Tenmarq is a hybrid wheat obtained from a cross between P1066, a Crimean wheat similar to Kanred, and Marquis. It should, therefore, have good baking qualities on the basis of inheritance. A comparison of the dough curves illustrating Tenmarq (see Figure 4) with the dough curves illustrating the hard red spring wheat flours (see Figure 2), and also the hard winter wheat flours (Figure 3), shows striking similarities. There are variations in the dough curves made from the different samples of Tenmarq flour but not any more so than in the dough curves made from different lots of hard red spring flour and well known hard red winter wheat flour. The Tenmarq wheats were grown in the same areas as the Turkey wheats whose doughs are illustrated in Figure 3. The general characteristics of the dough curves from the Tenmarq flours indicate that they should have a quality comparable to the flour milled from high quality hard red

spring and Turkey hard red winter wheats. Very extensive baking tests of Tenmarq flour made at Manhattan and by cooperating cereal chemists, have shown that this wheat is comparable in baking quality to Marquis or Turkey.

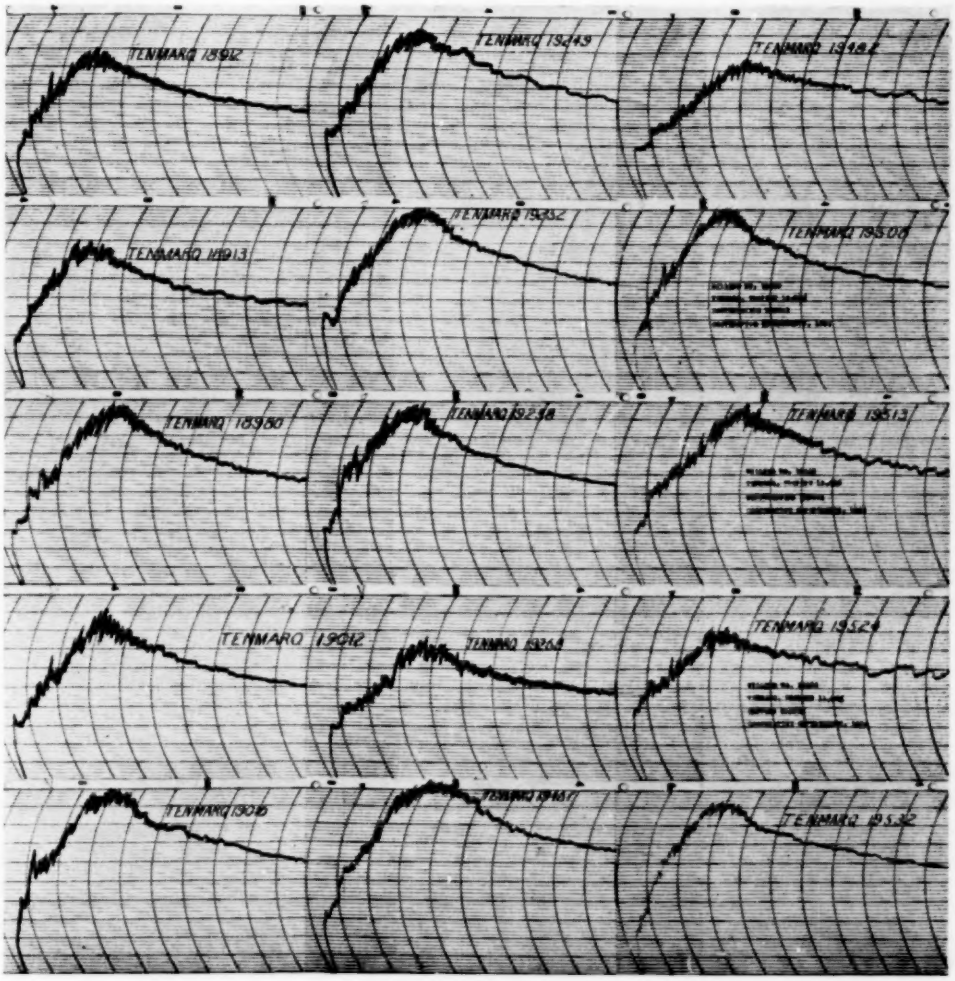

Fig. 4. Curves from flours milled from Tenmarq wheat, a hybrid between Kanred and Marquis, grown in 1933 and 1934. The localities were as follows:

Tenmarq, Manhattan, Kans.	Tenmarq, Composite, state.	Tenmarq, Manhattan, Kans.
Tenmarq, Manhattan, Kans.	Tenmarq, Manhattan, Kans.	Tenmarq, Southwest Kans.
Tenmarq, Hays, Kans.	Tenmarq, Manhattan, Kans.	Tenmarq, Northwest Kans.
Tenmarq, Northwest Kans.	Tenmarq, Composite, state.	Tenmarq, Eastern Kans.
Tenmarq, Southwest Kans.	Tenmarq, Hays, Kans.	Tenmarq, Manhattan, Kans.

The characteristics of these curves are very similar to those in Figure 2, spring wheats, and in Figure 3, Turkey wheats. These curves indicate that the qualities of Tenmarq wheat should be very similar to those of the spring and Turkey wheats. The similarities in quality between Tenmarq and the Turkey wheats have been confirmed by very extensive baking tests conducted in cooperation with numerous laboratories.

The Blackhull samples (*Figure 5*). The dough curves made from the Blackhull wheat flours are strikingly different from those shown for the preceding three groups. The time of development is, on the average, one-third shorter; the top is much less rounded; the most striking characteristic of all, however, is the sudden disappearance of the amplitude,

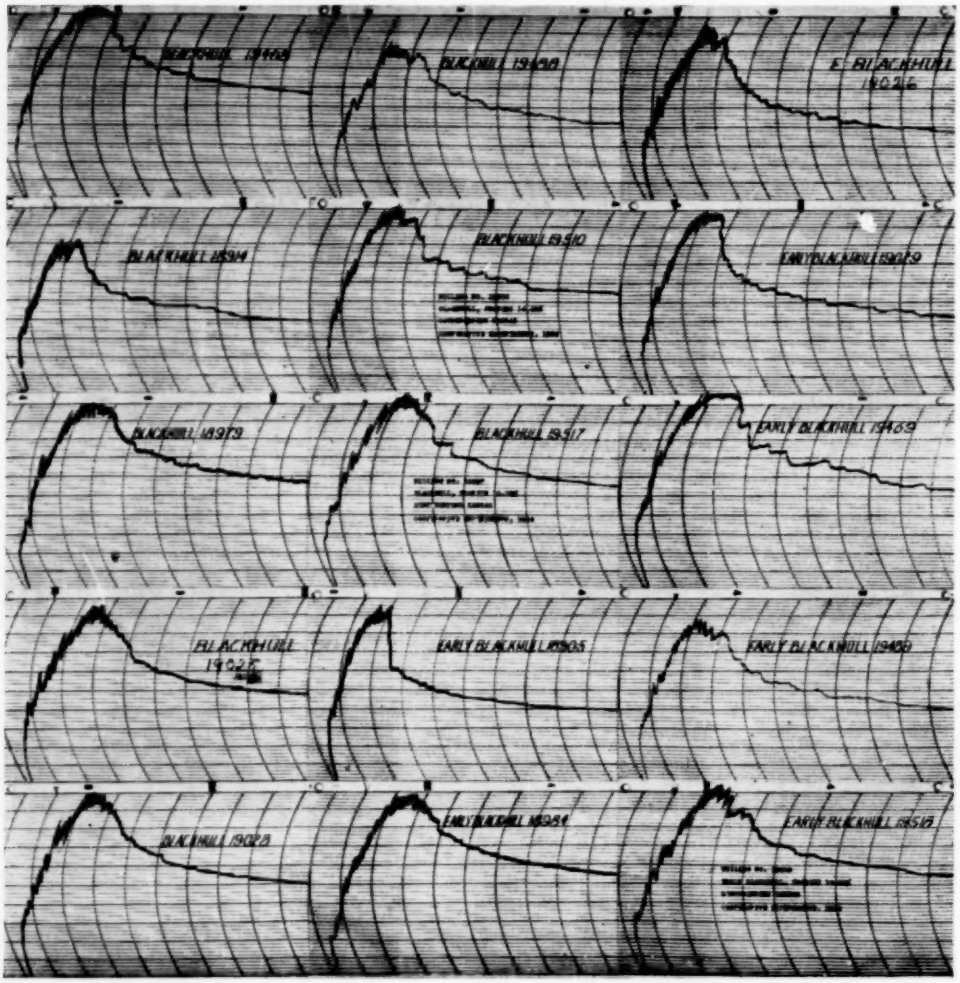

Fig. 5. Curves from flours milled from Blackhull and Early Blackhull wheats, grown in 1933 and 1934. The localities were as follows:

Blackhull, Hays, Kans.	Blackhull, Manhattan, Kans.	Early Blackhull, Northwest, Kans.
Blackhull, Manhattan, Kans.	Blackhull, Southwest, Kans.	Early Blackhull, Southwest, Kans.
Blackhull, Hays, Kans.	Blackhull, Northwest, Kans.	Early Blackhull, Hays, Kans.
Blackhull, Northwest, Kans.	Early Blackhull, Manhattan, Kans.	Early Blackhull, Manhattan, Kans.
Blackhull, Southwest, Kans.	Early Blackhull, Hays, Kans.	Early Blackhull, Northwest, Kans.

The characteristics of these curves are strikingly different from those shown in Figures 2, 3, and 4. The rate of development is more rapid, the peak is sharper, the descent is steeper, and the breakdown of the dough is more complete shown by the curves becoming smooth. These characteristics indicate that the handling properties of the doughs of these wheats are very different from Turkey and the spring wheats as well as Tenmarq.

resulting in a smooth line. The dip after the turn at the top is more steep than in the dough curves from Turkey or the hard red spring wheat flours. These general characteristics are as notable in flours milled from Early Blackhull, a newer variety, as in Blackhull which is better known. Baking tests have shown that there are no essential differences in the quality of these two strains of Blackhull. The larger to and from movements in some of the curves, notably No. 19469, are due to intermittent sticking of the dough to the bottom of the bowl and then tearing loose.

The quality of Blackhull wheat has been the subject of more controversy than that of any other hard red winter wheat. The dough curves shown in Figure 5 demonstrate that the behavior of the dough made from the flour of this wheat is fundamentally different in its reaction towards mechanical manipulation, than the dough made from flours milled from Turkey or the hard red spring wheats, wheats which were accepted as standards of quality when Blackhull came onto the scene. The high acre yields characteristic of Blackhull wheat, its dark color, the desirable physical appearance of the grain, and its test weight that averaged two pounds higher than Turkey, were the main factors which contributed to its rapid adoption by wheat growers in Kansas and the Southwest. Experience has shown that any new wheat which is fundamentally different from the old and accepted varieties will meet with criticisms, and these criticisms will continue until the trade has learned the characteristics of the new wheat and adapted their methods of milling and baking to meet these characteristics.

The characteristics of the dough curves shown in Figure 5 help to explain the basis for the criticisms of Blackhull. The steep rise of the curve shows that the development of the dough was more rapid than in Turkey wheat flours because of the easier wetting of the flour. Further, because of this rapid absorption of water, there was danger of using too much water and the dough becoming too slack. The use of high speed mixers was reaching its height when Blackhull wheat came onto the scene, and since Blackhull flour was sensitive to severe mixing there was also the danger of obtaining a sticky dough. Prominent cereal chemists and millers have made the statement that much of the trouble ceased when the mixing time was cut from one-third to one-half, and the time of fermentation was carefully adjusted to meet the needs of the flour milled from Blackhull wheat.

Cheyenne, Oro, and Cooperatorka (*Figure 6*). These three varieties represent newer wheats of the hard red winter class. The dough curves illustrating these wheats are strikingly different from those for Blackhull wheat. The rate of development is even much slower than in the

hard red spring or Turkey varieties. The tops of the curves are also more rounded, and their amplitude persists to the end, indicating no complete breaking down of the dough. From this it appears that they are not sensitive to prolonged mixing, and according to some workers this

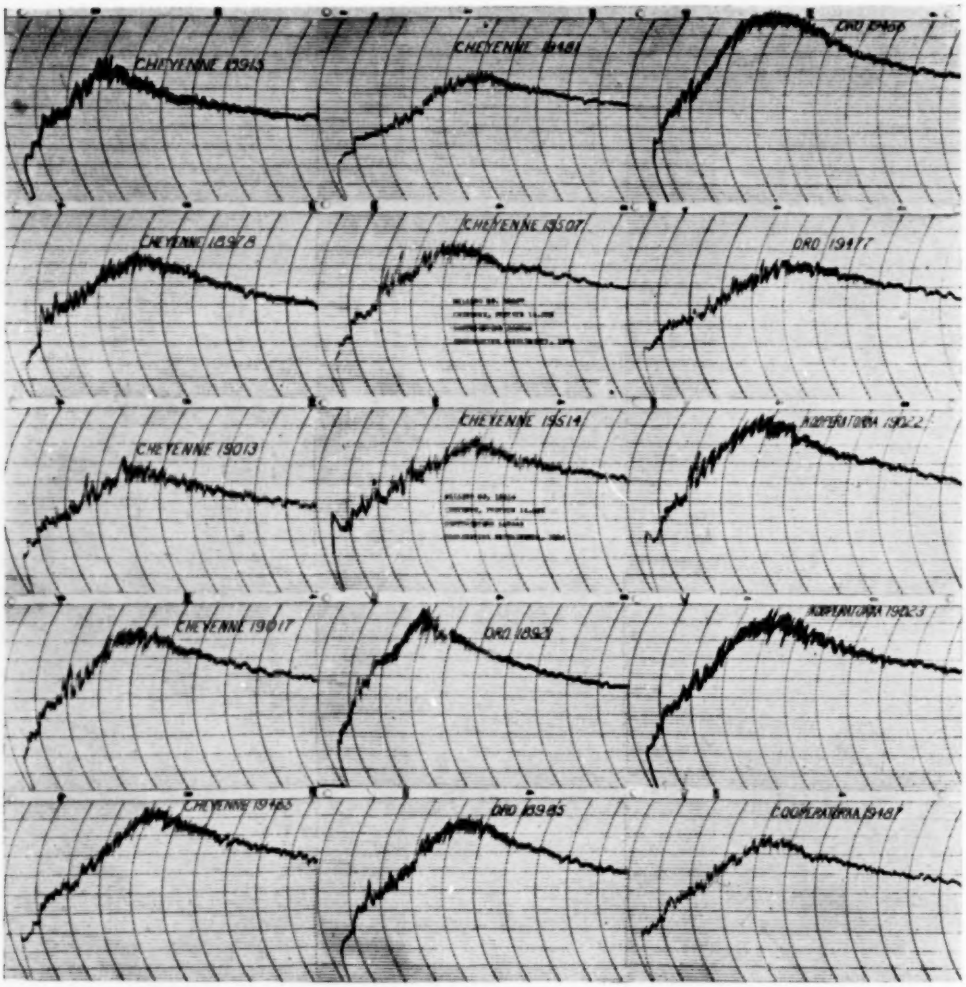

Fig. 6. Curves of Cheyenne, Oro, and Cooperatorka, comparatively new winter wheats. The location of these wheats grown in 1933 and 1934 were as follows:

Cheyenne, Manhattan, Kans.	Cheyenne, Manhattan, Kans.	Oro, Hays, Kans.
Cheyenne, Hays, Kans.	Cheyenne, Southwest, Kans.	Oro, Manhattan, Kans.
Cheyenne, Northwest Kans.	Cheyenne, Northwest Kans.	Cooperatorka, Northwest, Kans.
Cheyenne, Southwest Kans.	Oro, Manhattan, Kans.	Cooperatorka, Southwest, Kans.
Cheyenne, Hays, Kans.	Oro, Hays, Kans.	Cooperatorka, Manhattan, Kans.

The characteristics indicate a slow rate of dough development, and large resistance to prolonged mechanical action. The height of these curves was due to high protein content. The general characteristics are somewhat similar to the soft wheat cracker flour, except height, shown in Figure 1. This suggests that if these wheats would be low in protein, they could be used like those of soft red winter wheat.

means greater fermentation tolerance. This has not been established, partly because there is no adequate method of measuring fermentation tolerance. Too little work has been done on wheats of this type to have a satisfactory basis of interpretation of the curve characteristics in terms of baking values. As a rule the baking values of these wheats

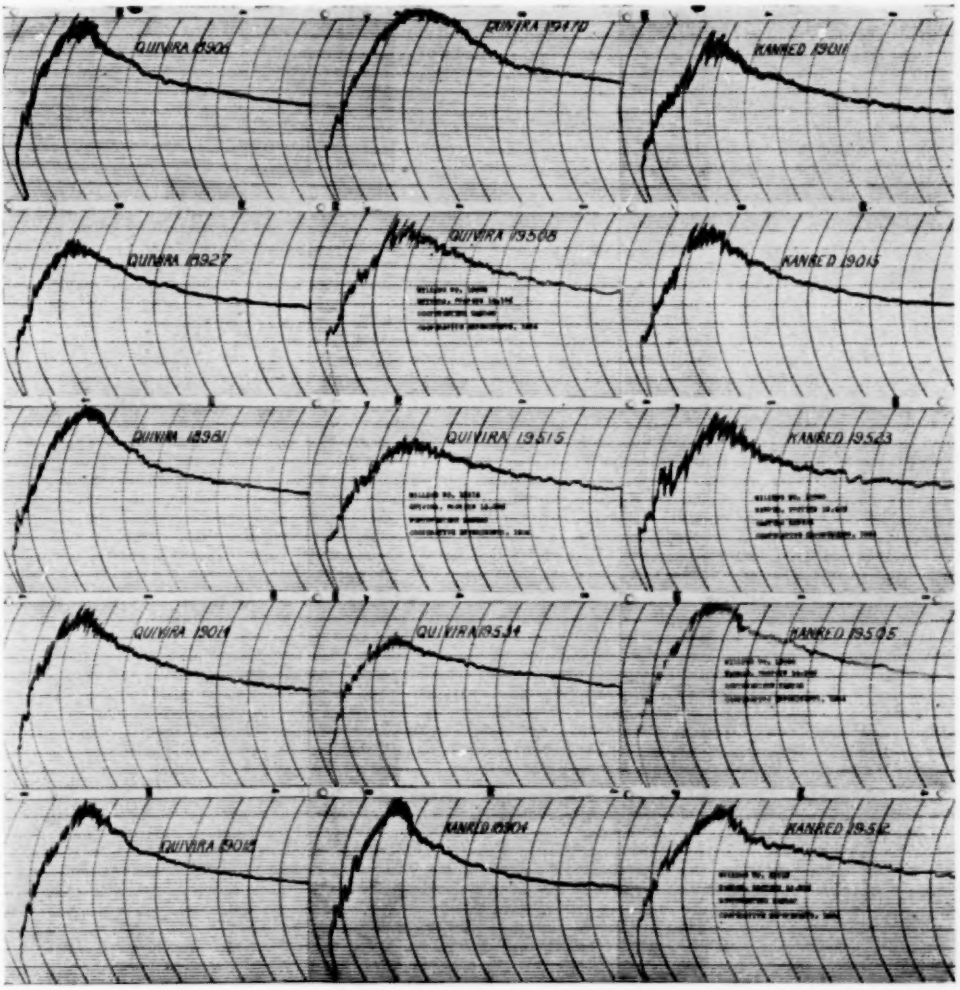

Fig. 7. Curves of Quivira, a new early winter wheat and the well known Kanred. The locations at which these wheats were grown in 1933 and 1934 were as follows:

Quivira, Manhattan, Kans.	Quivira, Hays, Kans.	Kanred, Northwest, Kans.
Quivira, Manhattan, Kans.	Quivira, Southwest Kans.	Kanred, Southwest Kans.
Quivira, Hays, Kans.	Quivira, Northwest Kans.	Kanred, Eastern Kans.
Quivira, Northwest Kans.	Quivira, Manhattan, Kans.	Kanred, Southwest Kans.
Quivira, Southwest Kans.	Kanred, Manhattan, Kans.	Kanred, Northwest Kans.

The curves of Quivira and Kanred show strikingly similar characteristics and indicate that the qualities of the new wheat Quivira would be very much like that of Kanred. These similarities in quality have also been confirmed in extensive baking tests.

have been found to be satisfactory. Oro has usually made an excellent loaf of bread. The results with Cheyenne have been more or less uncertain. On some samples the results are good and on others rather indifferent. This may be due to the baking method rather than inherent

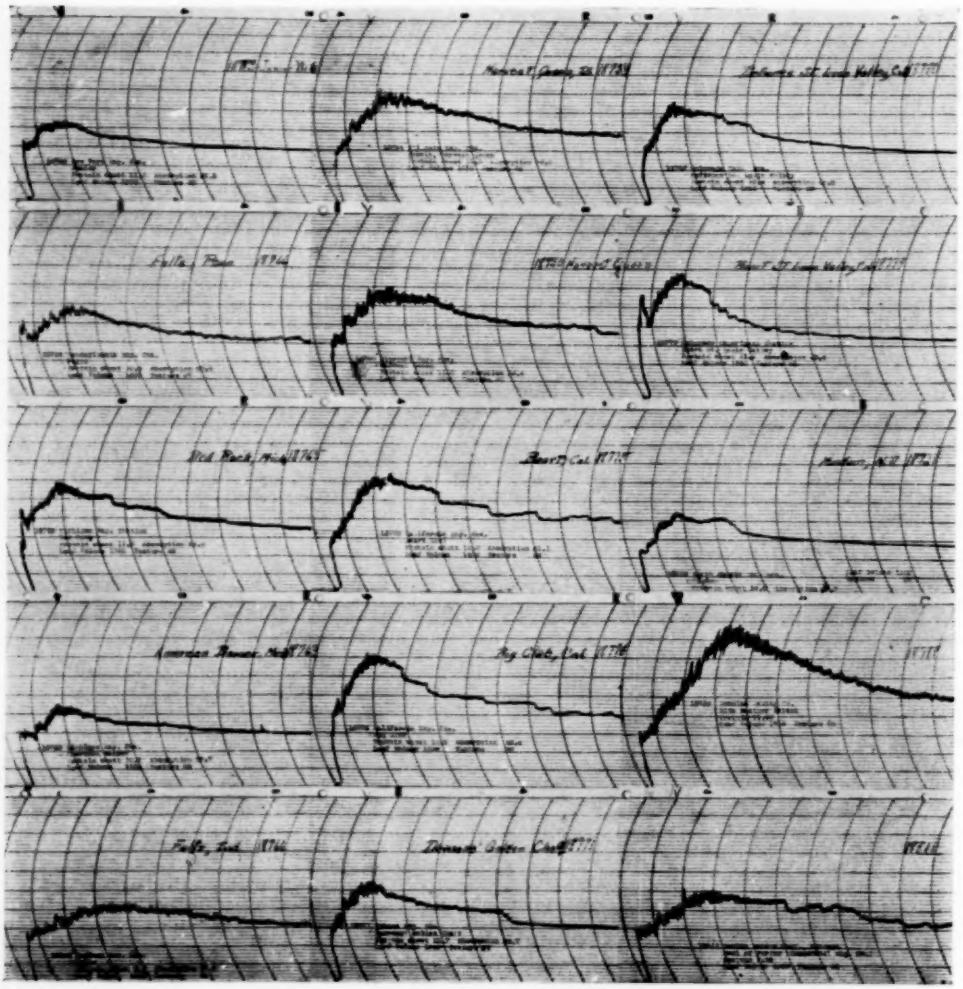

Fig. 8. Curves of soft wheat flours from several states. The location at which these wheats were grown in 1932 were as follows:

Junior No. 6, New York.
Fultz, Pennsylvania.
Red Rock, Michigan.
American Banner, Michigan.
Fultz, Indiana.
Harvest Queen, Illinois.
Harvest Queen, Missouri.
Baart, California.
Big Club, California.
Dawson's Golden Chaff, Ottawa, Canada.
Defiance, Colorado.
Baart, Colorado.
Mindum, North Dakota. (Durum for comparison.)
Hard wheat flour, Kansas City Bakery. (For comparison.)
Soft wheat flour, Chicago Bakery.

These curves are characteristically different from those of the hard bread wheats shown in Figures 2 and 3. The time for development is short, the tops are rounded and the breakdown is complete. These characteristics indicate the delicate gluten desired in baking with soda as the leavening agent. The unsuitability of Durum for bread making is shown in the curve from Mindum.

weaknesses in the variety. It is apparent from the dough curves that the handling of doughs of such wheats should be different from those of the Blackhull type.

Quivira and Kanred (*Figure 7*). Quivira is a new, early variety of hard red winter wheat developed by the Department of Agronomy, Kansas State College, in cooperation with the United States Department of Agriculture, in response to the demand for an early wheat, in order to extend the harvest period, especially where the combine is used. Quivira is the result of a cross between Kanred and Prelude. Nursery and plot tests have shown that it has some excellent agronomic qualities. Baking and other tests have shown that Quivira is not equal to Tennarq or to the hard red spring wheats, and perhaps note quite as good as the better Turkey types. Since Kanred, which is a Turkey type, is considered an acceptable wheat, it was thought best to compare Quivira with this variety. The general characteristics of the dough curves made from the flour of these two wheats are strikingly similar. The baking tests, not only in our laboratory, but in more than a score of other laboratories, have shown that in general, these two wheats have similar baking values.

Soft wheats grown in other states (*Figure 8*). Soft wheats were obtained from various experiment stations and milled on an Allis experimental mill. These dough curves may be taken as typical of those obtained from the flours used for biscuit, cracker, cakes, and other bakes where sodium bicarbonate is the leavening agent. For comparison there are included, lower right hand corner, a strong commercial bread flour, and a commercial pastry flour. A dough curve from a durum wheat flour, Mindum, is also included.

The main characteristics of these dough curves is their quick development, requiring about two minutes or less, in most cases. The tops of the curve are generally rounded, but probably the most outstanding characteristic of all is the breaking of the dough soon after development, resulting in a smooth line. The height of the top of the curve from the base line is also much lower than in bread wheats.

The wheats from New York, Michigan, and Pennsylvania are more uniform in the characteristics associated with soft wheat flour than the others. Harvest Queen from Missouri and Illinois shows more strength, indicating that the flours would be more of the nature of an all-purpose type, useful for both bread and pastry. The white wheats show as complete breaking down of the dough as the accepted soft red wheats. The protein of some samples was high and this raised the height of the curve. Durum flour shows the most complete breaking of all, indicating the unsuitable character of the dough for bread making.

Kansas soft wheat (*Figure 9*). Some of the Kansas soft wheat flour dough curves, notably those made from Fulcaster and Kawvale flours, resemble the dough curves obtained from hard wheat flours. Baking tests have also shown that these wheats are rather strong for soft flour purposes. Kawvale was selected from an eastern soft wheat

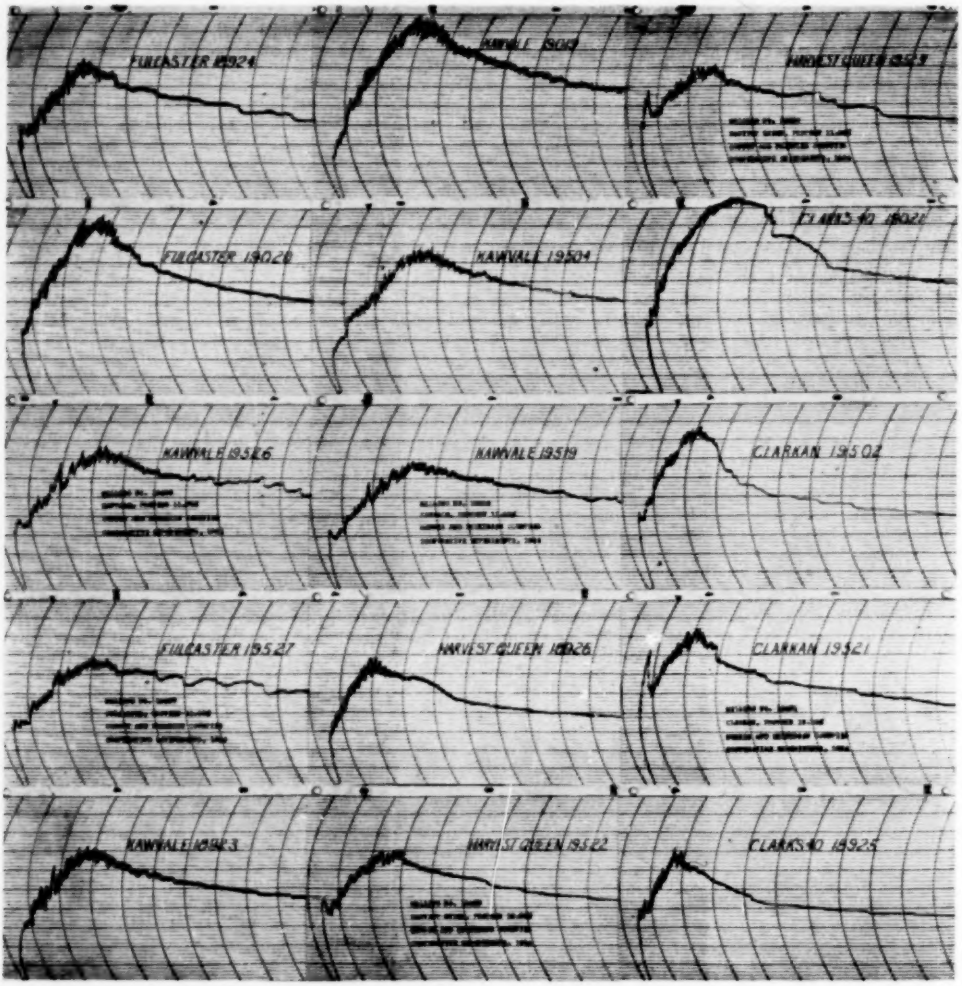

Fig. 9. Curves from soft wheats grown in Kansas. The locations at which these wheats were grown in 1933 and 1934 were as follows:

Fulcaster, Manhattan, Kans.
Fulcaster, Central Kans.
Kawvale, Southeast Kans.
Fulcaster, Southeast Kans.
Kawvale, Manhattan, Kans.
Kawvale, Central Kans.
Kawvale, Manhattan, Kans.
Kawvale, Central Kans.
Harvest Queen, Manhattan, Kans.
Harvest Queen, Central, Kans.
Harvest Queen, Southeast Kans.
Clarkan (Clark's 40), Central Kans.
Clarkan, Southeast Kans.
Clarkan, Central Kans.
Clarkan (Clark's 40), Manhattan, Kans.

The characteristics of these curves, particularly Kawvale, show that these flours were much too strong for typical soft wheat purposes. Part of this "strength" is due to the high protein content.

and is well adapted to eastern Kansas. The criticisms of this variety from the time it was first distributed have been that it was too strong to fill the requirements for soft wheat. Fulcaster has also been shown, in very extensive tests, to have a rather strong gluten, in some cases equaling that of the hard red winter wheats. Harvest Queen, however, shows more of the characteristics looked for in a soft wheat than either Kawvale or Fulcaster.

Clarkan is a soft wheat of the Blackhull type, bred by Earl G. Clark of Sedgwick, Kansas. It has the typical characteristics of Blackhull, but in a soft wheat these are not undesirable. Hence, where Clarkan can be grown so as not to have too high a protein, as in eastern Kansas, it will be acceptable as a soft wheat.

General Discussion and Summary

In wheat improvement it is necessary to establish certain standards. Flours used for specific purposes like bread baking, cracker, biscuit or cake making, may be selected as standards. The hard red spring wheat, Marquis, and the hard red winter wheat, Turkey, have established the standards desired for the bread wheats. The gluten in these wheats has characteristics which are particularly desired in bread baking with yeast as the leavening agent. Two very important physical characteristics in bread doughs are expansibility which allows for the expansion due to the production of carbon dioxide and the elasticity which prevents too easy expansion. If these two exist in a satisfactory balance, the properly fermented and proofed dough will produce, when baked, a light bread which meets accepted standards. Other factors must also be satisfactory, particularly gas production, but these are presupposed in the present discussion.

Some recent physical devices for testing desirable characteristics in dough include: The Chopin Extensimeter, the Bühler Comparator, the Brabender Farinograph, and the Swanson-Working Recording Dough Mixer. The first two are designed to test mainly the elasticity or the extensibility of dough. The last two are designed to test mainly the rate of dough development and behavior of dough under the stress of extended mechanical action. By comparing dough curves or data obtained on dough from flours accepted as standards, with those obtained from new wheats, the characteristics of these wheats may be judged.

Characteristics of the dough curves made on the Swanson-Working Recording Dough Mixer from the flour of a hard red spring wheat such as Marquis, and a hard red winter wheat such as Turkey, are selected as standards for bread wheats. The curve from a bread dough made

from a strong hard red spring or hard red winter wheat flour rises gradually, taking about three minutes to reach the top, which is in the upper half of the graph paper. The top or peak of the curve is more or less rounded, and the amplitude decreases gradually, but never entirely disappears.

Dough curves obtained from Marquis flour as well as from flour milled from some of the newer varieties of spring wheats are very similar to the dough curve obtained from a hard spring wheat bread flour of known baking value. Dough curves obtained from flours milled from Turkey wheats grown at various places are similar to the dough curves obtained from spring wheat flours. The dough curves obtained from the flour milled from various samples of Tenmarq wheat demonstrated that this variety has dough characteristics strikingly similar to the hard red spring and to the Turkey wheats. The dough curves made from the Blackhull group have very marked differences when compared with the dough curves characteristic of the varieties Marquis, Turkey, or Tenmarq. The steep rise of the curve shows that the dough develops more rapidly as the curve makes a quick turn downward from the peak and the amplitude persists but for a short while. These phenomena indicate a high sensitivity to severe mixing and that under extended mixing that Blackhull dough breaks down completely. The physical characteristics of gluten from Blackhull wheats are therefore fundamentally different from those of the hard red spring and Turkey types.

Flours made from Cheyenne, Oro, and Cooperatorka wheats have dough curves, except for height, more like those of a cracker flour. If these wheats were low in protein for the hard wheats, dough curves would be very much like the curves for the medium protein soft wheat flours.

Quivira is a new early maturing winter wheat variety. The dough curves for this variety are very similar to those for Kanred flour. The baking tests with Quivera flour have also shown similarity in baking value to Kanred flours. The dough curves made with the soft wheat flours from states further east, when compared with the dough curves made from Kansas grown soft wheat flours show that the latter are too much like bread wheat flours and are not as well adapted for typical soft flour purposes. The tests show that Kawvale has more of the characteristics desired in a hard wheat than in a soft wheat.

The indications given by these dough curves should be supplemented by other tests. The evidence so far is that when the dough curves of a new wheat flour are like those of a typical spring wheat flour such as Marquis or of a typical hard winter wheat flour such as Turkey, the flour will prove satisfactory as a bread wheat flour. The dough curves

of new soft wheat flours should be compared with those obtained on flours used for specific purposes such as cake, cracker, and biscuit baking.

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BOUND WATER IN BREAD MAKING[1]

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Bound Water and Its Properties

The problem of water in colloidal systems has long been the object of investigation. Investigations dealing with this problem were conducted as early as the beginning of the last century. Vogel in 1820 investigated the influence of freezing on the state of water in wheat starch. Since that time a great many papers dealing with the condition and importance of water in colloid systems have been published. Special attention has been paid to this problem during the last 10 to 15 years.

The water associated with the biocolloids plays an exceptionally important role, being from the point of view of percentage weight as well as from the standpoint of physiology one of the major constituents of all living organisms.

All biocolloids are highly hydrophilie and undergo marked changes in properties upon hydration or dehydration. The biocolloids in water do not form a homogeneous system because of the various forms of bonds existing between substance in a colloidal state on the one hand and water on the other. Kuhn (1924) distinguishes seven forms in which water may exist, *i.e.*, occluded, adsorbed, capillary, colloidally fixed, chemically combined, osmotic, and complex-bound. Ostwald (1921) who had investigated water in peat, pointed out the following forms of water condition: occluded, capillary, colloid, osmotic and chemically combined.

Polanyi (1920), Newton and Gortner (1922), and other investigators (Marinesco, 1931; Reinke, 1879; Gortner and Harris, 1914; Newton, 1922, 1923, 1924; Newton and Martin, 1930; Robinson, 1928) have considered that water existed in two principal states—free water and bound water. The free water in colloid systems is the intermicellary liquid and does not essentially differ from water as we know it in bulk. The bound water is that fraction which has become, for the time being, an integral part of the colloid particle. As is well known, the molecules of water are dipoles. The colloid particles, possessing a certain charge attract the water molecules and produce a definite orientation of the

[1] Contribution from the Laboratory for Colloid Physics and Chemistry of the Scientific Research Institute for the Baking Industrie of the U. S. S. R., Moskow.

latter. In this way the adsorption of water takes place. The colloid particles strongly attract the first layer of water molecules. This attractive force falls off at greater distances from the colloid particles so that the molecules in successive shells of water are held more and more loosely and at a certain distance from the colloid particles we have water in a free state. Thus the transition from bound water to free water is gradual, not abrupt. Lipatoff (1926) tried to determine the thickness of the water layer adsorbed on a colloid particle. He calculated from viscometric data that for Geranin G dyestuff the colloid particle of 4.9×10^{-8} cm. diameter is surrounded by a layer of water 17.6×10^{-8} cm. thick. The total radius of the particle with the water stratum is equal to 22.5×10^{-8} cm.

Bound water possesses many interesting peculiarities. According to Ewing and Spurway (1930) the density of fixed water is 1.027 to 1.0285 which corresponds to the density of water under the pressure of 750 atmospheres. According to Marinesco (1931) bound water is held by still higher pressures of the order of ten thousands atmospheres. Tammann (1910) and Bridgman (1912) have shown that water under great pressure may exist in solid condition even at a temperature above zero. "Ice VI" has been shown to be stable at these high pressures up to $+76.55°$ C. This fact has lead workers to conclude that bound water may be considered as a solid. Reinke (1879), Newton (1922, 1923), Newton and Martin (1930), Newton and Gortner (1922), Gortner (1929, 1930), and Gortner and Harris (1914) point out that owing to the considerable force with which water is held by the colloid particles, bound water may be expressed from the biocolloids only with great difficulty. It differs from free water by its inability to pass freely through a living cell in the course of osmosis. Bound water practically does not dissolve even such solutes as sucrose which is easily dissolved in free water. Besides, bound water is characterized by a very low dielectric constant which, according to Marinesco (1931) is 2.2 as compared to 81 for bulk water. A number of other investigators (*cf.* Jones and Gortner, 1932, for literature citations) have demonstrated some farther properties of bound water such as resistance to freezing, peculiarities in light absorption, etc.

The problem of bound water in biocolloids is also of great significance in that it plays a highly important role in various physiological and pathological processes (Gortner, 1929, 1930; Balcar, Sansum, and Woodyatt, 1919; Thoenes, 1925; and Robinson, 1928). Thus the problem of old age appears to be closely connected with the change in the ratio of free to bound water in a body (Thoenes, 1925).

A series of researches by Newton and Gortner (1922), Newton

(1922, 1923, 1924), and Newton and Martin (1930) has shown that the winter-hardiness of wheats is due to a considerable degree to their capacity of binding-water. The drought resistance capacity of plants may be explained in the same way (Newton and Martin, 1930).

Bread baking is primarily a process of working up cereals. The problem of bound water in bread making is therefore of a great theoretical and practical interest. Newton and Cook (1930) apparently made the first researches in this field.

Principal Methods for Determining Bound Water

At the present stage, several methods based on one of the characteristic properties of bound water have been suggested for determining the amount of bound water in a given system.

1. The dilatometric method is one of the early methods proposed by Foote and Saxton (1916, 1917, 1917a) and improved by Jones and Gortner (1932). The method is based on the principle of measuring the expansion of water on freezing. Free water freezes close to zero degrees Centigrade. The amount of free water is therefore determined dilatometrically by measuring the volume change of a specimen upon freezing. The total content of water in the given sample being known, the content of bound water may be easily calculated.

2. Calorimetric method. Thoenes (1925) utilized Rubner's suggestion of using a a calorimeter for the determination of bound water. The sample with a known amount of water is cooled to — 20° C. whereupon free water alone freezes. The sample is then transferred to a water calorimeter. The change of temperature of the water in the calorimeter can be calculated in terms of the calories necessary for heating the whole mass and for melting the frozen free water. Thoenes (1925) as well as Robinson (1928, 1931) proposed formulae for calculating the quantity of bound water from the data so obtained.

3. The cryoscopic method was proposed by Newton and Gortner (1922). The method is based on the idea that bound water is not available as a solvent. Therefore if we add a certain quantity of sugar to a sol in which the total water content is known, the concentration of the solution obtained will be in excess of that expected as the sugar is not dissolved in the whole mass of water but only in that part which is free. Having determined the depression of the freezing points of the original sol and the one to which sugar has been added, the amount of bound water may be calculated by a special formula (*cf.* also Gortner and Gortner (1934).

4. The refractometric method has been suggested by Dumanski (1933, see also Dumanski, Kuhlmann and Golossowa, 1934, 1934a) and

is also based on the principle that water is not a solvent. The concentration of a sol before and after adding sugar is determined by means of a refractometer. The amount of bound water may be calculated by a special formula in terms of the increase in sugar concentration as compared with that which could be otherwise expected.

5. A polarimetric method has likewise been suggested by Dumanski (1933) and differs from the refractometric method in that the excess concentration of the solution is determined by a polarimeter.

Besides the above methods of determining the amount of bound water there are also a number of others, such as the viscometric technic, application of Stokes' Law (Dumanski, *et al.* 1913, and Marinesco, 1927) the dielectric polarization method (Marinesco, 1931), the fluorescence extinction method (Banow and Jakowlew, 1930) and others.

Experimental

In the present work Dumanski's refractometric method (Dumanski, 1933) has been adopted. The method was especially modified by the authors for flour, dough, and bread. The following procedure was adopted after the making of a series of preliminary experiments.

The weight of the material for study was calculated taking into account its average moisture content so that in every case 5 g. of absolutely dry matter was used. 50 g. of water were then added including the amount of moisture already present in the sample. After standing until solution equilibrium had been attained a known amount of the sugar was added to one of the samples. The latter were next shaken up and centrifuged. The content of solutes in the samples with and without sugar were determined by means of a refractometer. Calculation was carried out by the following formula:

$$x = A - \frac{Ab}{100-b} \cdot \frac{(100-b_2)\ (100-b_1)}{100\ (b_2-b_1)}$$

A = amount of added water in grams;
b = percentage content of sugar as dissolved in chemically pure water taken in a quantity equal to the total content of water in the sample;
b_1 = amount of solutes in the suspension as determined by the refractometer;
b_2 = amount of solutes in the mixture; suspension + sugar added;
x = amount of bound water in grams.

It must be noted that the above method was used to determine the power of biocolloids to bound water under certain conditions of excess moisture.

In the present work the water-binding capacity of various classes of flour has been studied as well as the dynamics of this property in the process of bread baking and its staling, and also the relationship between the loss in baking and of the drying of bread.

Investigation of the Capacity of Different Grades of Flour to Bind Water

The investigation of the capacity of various classes of flour to bind water yielded the data shown in Table I.

TABLE I

THE WATER-BINDING CAPACITY OF DIFFERENT FLOUR VARIETIES

Class of flour	Capacity of flour colloids to bind water
	%
Potato flour	29.4
Soft wheat flour	47.8
Durum flour	51.8
Oat-meal	45.8
Buckwheat flour	47.8
Barley flour	51.8
Corn flour	55.0
Rye flour	62.0
Soya flour	130.4

The results enable us to arrange all the classes of flour tested according to their water-binding capacity in the following order: Soya, rye, corn maize, durum wheat, soft wheat, potato flours.

The protein content of all the samples of the above classes of flour was determined by the Barnstein method as certain relationships exist between the protein content and the water-binding capacity. No complete parallelism, however, has been observed owing, undoubtedly, to the fact that the water-binding capacity of flour is influenced by the nature of the protein and the hydrophilic nature of the other components of the flour. In particular, rye starch is considerably more hydrophilic than is wheat starch; the former has a greater water-binding capacity, 78 to 80%, whilst the latter ranged from 40 to 42%.

It is well known that for preparing dough of a certain consistency, various classes and grades of flour require different amounts of water. This amount was determined by taking special weighed samples and is expressed in percent with respect to the weight of the flour. It is called "water-absorbing capacity." We have compared this property for some wheat flours with the bound water of the flour colloids. The data obtained are given in Table II.

TABLE II

BOUND WATER AND WATER-ABSORBING CAPACITY OF VARIOUS GRADES OF WHEAT FLOUR

Class of flour	Yield of flour [1]	Bound water	Water absorbing capacity [2]
	%	%	%
Winter wheat flour	75	46.4	50.0
" " "	85	48.4	54.1
" " "	96	54.4	62.8
Durum wheat flour	75	46.4	70.4
" " "	85	50.6	73.5
" " "	96	52.2	78.0
Spring wheat flour	75	44.4	60.2
" " "	85	46.4	66.0
" " "	96	48.4	64.0

[1] The yield of flour is calculated in percentage of the total amount of grain.
[2] Data of A. I. Lose (Technological Section of the Scientific Research Institute for Bread Baking Industry of the U. S. S. R.).

As the table shows, there is a direct relationship between the water-absorbing capacity of the flour and its water-binding capacity. The same relationship is, however, shown to exist between the water-binding capacity of the flour and its yield. This relationship also evidently has some connection with the greater bran content of low grade flour. Wheat flour bran's water-binding capacity ranges from 56 to 58% whereas for whole flour with sifted bran the water-binding capacity is about 45%. This case is undoubtedly connected with a higher protein content in low-grade flours.

The Dynamics of the Water-binding Capacity in the Bread-baking Process

A series of laboratory baking tests made on two grades of wheat flour (85% and 65% yield) and one grade of rye flour (96% yield) was made for the purpose of studying the dynamics of the capacity of flour to bind water in the bread-baking process.

The graphs illustrating the baking tests and the stages at which the samples were taken, as well as the actual data obtained, may be seen in Figures 1, 2, and 3 and in Tables III, IV, and V. It is clear from these data that flour colloids at the moment of transition to dough, undoubtedly increase their water-binding capacity. No appreciable change in water-binding capacity is to be observed in the process of dough formation of wheat flours. In the case of rye flour, however, at the moment of placing the bread in the oven, a slight increase is noticeable. During baking the water-binding capacity sharply increases. Scalding

produces a sharp increase in the water-binding capacity for wheat flour, as well as for rye flour, and this capacity is retained at all stages of the bread-baking process. A still higher water-binding capacity, which is preserved at all stages of the process, is to be noted in the case of rye flour. The method of bread making has a marked influence on the water-binding capacity of the flour; for instance, the bread colloids

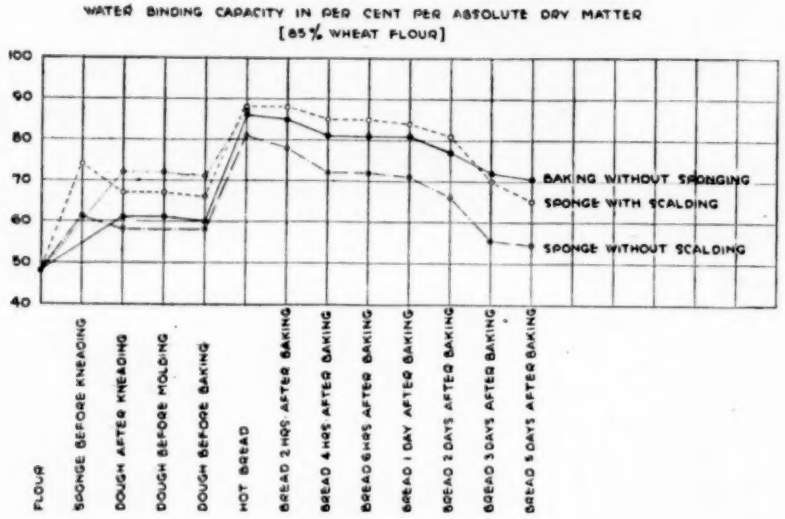

Fig. 1.

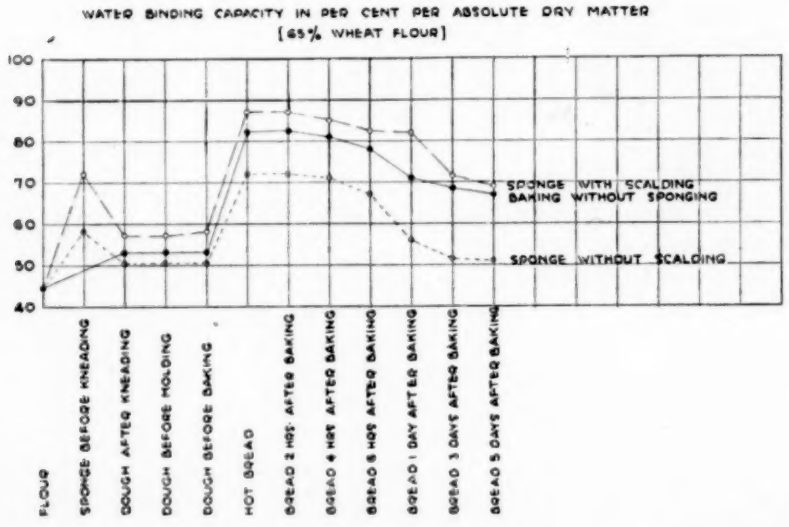

Fig. 2.

made by the straight-dough method binds more water than does bread made by the sponge-dough method.

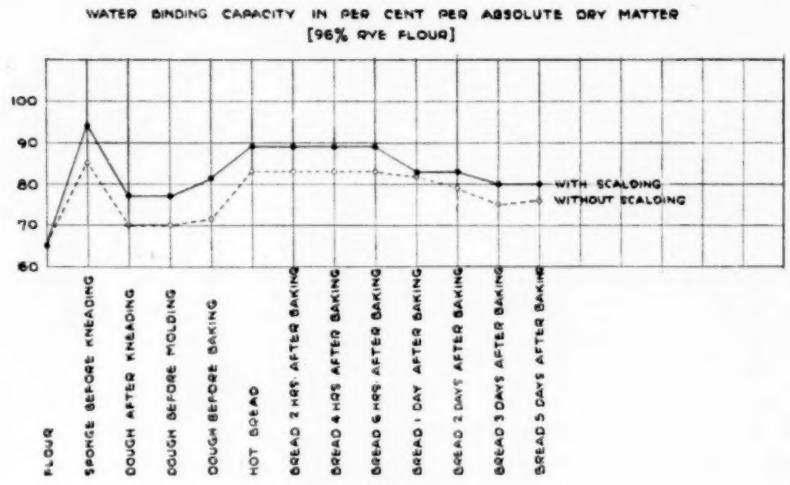

Fig. 3.

TABLE III

WATER-BINDING CAPACITY IN PERCENT OF ABSOLUTE DRY MATTER USING AN 85% WHEAT FLOUR

Material studied	Baking without sponging dough	Baking with scalding dough without sponging	Sponge without scalding	Sponge with scalding
Flour alone	48.00	48.00	48.00	48.00
Sponge before kneading	—	—	62.50	74.88
Dough after kneading	60.80	72.20	59.79	67.55
Dough before moulding	60.80	72.20	59.79	67.55
Dough before baking	60.00	70.93	59.79	67.14
Hot bread	87.23	86.83	81.06	87.29
Bread 2 hours after baking	85.20	—	79.17	87.29
Bread 4 hours after baking	81.60		73.12	85.31
Bread 6 hours after baking	81.60		73.12	85.31
Bread one day after baking	81.22		71.63	83.40
Bread 2 days after baking	77.84		66.66	81.76
Bread 3 days after baking	72.59		56.80	70.18
Bread 5 days after baking	72.57		55.37	66.17

Changes in Water-binding Capacity in the Staling of Bread

Boussingault (1852) stated at an early date that the staling process of bread is no way connected with its drying. The staling of bread appears to be a typical instance of the process of "aging" of a colloid gel. A change of the ratio of free to bound water, involving a decrease of the latter, occurs in the course of staling.

TABLE IV

WATER-BINDING CAPACITY IN PERCENT OF ABSOLUTE DRY MATTER USING A 65% WHEAT FLOUR

Material studied	Baking without sponging dough	Sponge without scalding	Sponge with scalding
Flour alone	44.00	44.00	44.00
Sponge before kneading	—	59.00	72.17
Dough after kneading	53.47	50.18	57.73
Dough before moulding	53.47	50.18	57.73
Dough before baking	53.47	50.18	58.33
Hot bread	82.71	72.16	87.20
Bread 2 hours after baking	82.71	72.16	87.20
Bread 4 hours after baking	81.22	70.39	85.20
Bread 6 hours after baking	79.60	67.36	83.53
Bread one day after baking	71.96	56.55	83.40
Bread 3 days after baking	69.72	52.69	70.74
Bread 5 days after baking	67.40	52.69	68.00

TABLE V

WATER-BINDING CAPACITY IN PERCENT OF ABSOLUTE DRY MATTER USING A 96% WHEAT FLOUR

Material used	Without scalding	With scalding
Flour alone	66.00	66.00
Sponge before kneading	84.87	94.39
Dough after kneading	70.24	77.67
Dough before moulding	70.24	77.67
Dough before baking	72.62	82.56
Hot bread	84.52	89.30
Bread 2 hours after baking	84.52	89.30
Bread 4 hours after baking	84.52	89.30
Bread 6 hours after baking	84.52	89.30
Bread one day after baking	82.56	83.04
Bread 2 days after baking	79.32	83.04
Bread 3 days after baking	76.22	79.15
Bread 5 days after baking	76.30	79.27

A decrease in the water-absorption of bread in the process of staling was noticed by Balland, as far back as 1894.

After a series of carefully performed experiments the same conclusion was arrived at by Lehmann (1894). Both investigators, however, failed to trace the mechanism of the phenomenon.

Figures 1, 2, and 3, and Tables III, IV, and V show that the water-binding capacity of bread decreases with staling.

Various methods of bread making produce distinct characteristics of the staling process. In the case of a sponge dough accompanied by scalding a sharp decrease in water-binding capacity may be observed on the third day, while with bread made by the straight-dough method

shows an almost immediate marked decrease in its water-binding capacity.

If the bread is made of dough without scalding a decrease will be noticed in four hours after removal from the oven.

The decrease in water-binding capacity of rye bread during staling proceeds more gradually than is the case of wheat breads.

At the present time there have been developed the following principal methods for a quantitative determination of the staling process: (*a*) A method for determining the variation of swelling capacity of bread in water (during the staling a decrease of this capacity is observed). This method has been suggested by Katz (1933 and 1933a) and modified by Peper (see Katz, 1933b). (*b*) A method for the determination of the hardening of the crumb with the staling of the bread which is measured by Verbeek-Katz's apparatus (see Katz, 1933a). The method consists in the penetration of a plate of a definite diameter into the crumb, under standard conditions. (*c*) A method for determining the quantity of soluble polysacharides in water (this quantity decreases with the staling of the bread). This method has also been suggested by Katz (1933a). (*d*) A method based on viscosity changes in bread suspensions (with the staling of the bread the viscosity decreases). The method has been suggested by Karacsonyi (1929). This method is very interesting and deserves the greatest attention. It is intimately connected with the degree of hydration of the colloid particles. From this point of view the refractometrical measurement of the water-binding capacity of bread during staling may serve as a new, simple, and rapid method for the quantitative control of the staling process.

Here the following fact may also be mentioned: Rusks prepared by drying bread at 105° C. generally show almost the same bound-water capacity as freshly baked bread, while rusks dried at room temperature in a desiccator over sulphuric acid show a considerably reduced water-binding capacity.

Water-binding Capacity and Loss in Baking

The loss in baking is the difference between weight of dough when entering the oven and the weight of the bread as it leaves the oven. The greatest part of the loss in baking is caused by the vaporization of water. Undoubtedly, the amount of vaporized water is dependent on the capacity of the dough colloid to bind water. To investigate this relationship laboratory baking tests were made in two ways: (*a*) the colloids of the various grades of flour were exposed to different conditions of moisture, corresponding to their water-absorption capacity, taking into account

the main features of the process (*i.e.*, as is usual in practice); (*b*) colloids of various kinds of flour were placed under identical moisture conditions (*i.e.*, the same ratio between the amount of absolutely dry flour and water was kept for all grades of flour).

The data obtained by the first method are given in Table VI.

TABLE VI

THE DEPENDENCE BETWEEN THE WATER-BINDING CAPACITY AND LOSS IN BAKING

Method of baking	Water binding capacity in grams per 100 grams of absolute dry matter		Loss in baking	Gain in weight
	Dough	Hot bread		
	Grams	*Grams*	%	%
	Sponge-dough method			
Sponge	51.63	63.04	8.9	43.2
Sponge-dough with scalding	60.62	69.78	6.5	44.2
Sponge with maltose	67.80	73.75	5.2	43.6
Sponge with whey	71.63	76.74	8.2	45.8
	Straight-dough method			
Straight-dough	56.87	68.72	9.2	43.9
Scalding without sponge	60.83	72.17	6.1	48.8
Straight-dough with maltose	70.00	75.02	5.1	45.1

The results given in Table VI show that the higher the water-binding capacity of the dough colloids, the lower is the water evaporated during baking and the greater is the gain in weight. The factors which increase the water binding capacity of dough colloids decrease loss in baking and increase gain in weight.

In Table VII the same data are given but calculated to 100 g. of dough water.

TABLE VII

THE DEPENDENCE BETWEEN THE WATER-BINDING CAPACITY IN DOUGH AND THE QUANTITY OF EVAPORATED WATER DURING THE BAKING PROCESS

Method of baking	Water binding capacity in grams per 100 g. of dough water	Amount of vaporized water in grams per 100 g. dough water
	Grams	*Grams*
Sponge-dough method	63.30	20.7
Sponge-dough with scalding	78.70	14.9
Sponge-dough with maltose	96.96	12.7
Sponge-dough with whey	92.43	14.8
Straight-dough method	72.53	21.0
Straight-dough with scalding	81.20	13.8
Straight-dough with whey	98.60	12.2

The data given in Table VII confirm more clearly the stated dependence.

The results obtained by the second method of laboratory baking test are summarized in Table VIII.

TABLE VIII

THE DEPENDENCE BETWEEN THE WATER-BINDING CAPACITY IN DOUGH AND BREAD AND THE QUANTITY OF EVAPORATED WATER DURING THE BAKING PROCESS

Flour samples Nos.	8	9	12	—	—
Method of bread making	Without scalding	Without scalding	With scalding	Without scalding	With scalding
A. Sponge-dough methods					
Water-binding capacity of dough—*grams per 100 grams of absolute dry matter*	63.70	65.90	69.80	66.40	76.70
Water-binding capacity of hot bread—*grams per 100 grams of absolute dry matter*	69.30	76.10	79.50	76.20	80.45
Loss in baking—*grams per 100 grams of absolute dry matter*	19.20	17.80	15.50	17.80	17.00
Amount of vaporized water—*grams per 100 grams of scalded dough*	22.60	21.00	18.30	21.00	20.10
Possible amount of bound water—*grams*	70.40	76.00	80.50	77.40	88.60
B. Straight-dough methods					
Flour samples Nos.	8	9	10	11	12
Water-binding capacity of dough—*grams per 100 grams of absolute dry matter*	55.10	70.60	72.40	63.90	72.40
Water-binding capacity of hot bread—*grams per 100 grams of absolute dry matter*	71.60	80.45	80.45	75.90	78.20
Amount of vaporized water—*grams per 100 grams of absolute dry matter*	37.30	23.60	23.10	26.20	23.00
Average gain in weight according to data of Technological Section—*grams*	33.10	42.20	38.20	36.80	39.30

From these results it may be seen that under the same conditions of moisture, that flour which has the greater capacity for binding water releases it in baking with the greatest difficulty. The scalding both increases the water-binding capacity and reduces loss in baking. There seems also to be a direct connection between the water-binding capacity and the average gain in weight.

Water-binding Capacity and Loss in Bread Drying

A certain amount of both water and volatile matter vaporizes as hot bread is cooled. At the same time there begins in the bread the process of colloid "aging" which reduces water-binding capacity. Thus the relative quantity of free water in bread increases and this phenomenon has a direct effect upon the amount of water evaporated during storage (loss in drying). Generally speaking, the magnitude of the loss in drying depends upon a number of working conditions, such as the method of storage, temperature, and moisture conditions in storehouse, the type of ventilation, etc. However, other conditions being equal the water-binding capacity of colloids is one of the main factors governing the amount of water vaporized in storage. To study this question, a series of laboratory baking tests according to different methods was carried out. The bread was stored under laboratory conditions and was weighed at definite intervals. Simultaneously with weighing, samples were taken to determine the water-binding capacity of the bread colloids. The data obtained are given in Table IX.

TABLE IX

THE WATER-BINDING CAPACITY AND LOSS IN BREAD DRYING

	Water-binding capacity—*grams per 100 grams of absolute dry matter*					Loss in drying—*grams per 100 grams of freshly baked bread*			
	Hot bread	in 1 day	in 2 days	in 3 days	in 5 days	1 day	2 days	3 days	5 days
Sponge	68.04	59.8	57.55	56.2	51.9	8.5	12.4	16.2	20
Sponge+scalding	69.78	66.78	—	—	—	7.3	—	—	—
Sponge+maltose	73.75	70.4	—	—	—	6.1	—	—	—
Sponge+whey	76.74	70.0	70.0	67.8	60.8	5.0	9.2	11.9	16

These data show that the higher the water-binding capacity of the bread colloid, the less is the loss in drying. This relationship appears to be very striking even on the first day of storage. After prolonged storage the effect is markedly increased. The factors increasing water-binding capacity (maltose, butter-milk and scalding) reduce the amount of loss in drying.

In conclusion we wish to acknowledge and express our thanks to Prof. A. W. Dumanski, under whose initiative the work was carried out.

Conclusions

Water-binding capacity is an important physico-chemical index characteristic for colloids, dough, and bread.

According to water-binding capacity the tested classes of flours stud-

ied can be placed as follows: soya, rye, corn (maize), durum wheat, soft wheat, potato flours.

Separate components of flour show quite different water-binding capacities (in particular rye starch is more hydrophilic than is wheat starch).

The water-binding capacity of various flours is to a great extent determined by the protein content of the flour.

A direct connection between the water-absorption capacity of flour and its water-binding capacity has been shown.

There exists a similar connection between flour yield and its water-binding capacity.

Generally speaking the colloids of sponge, dough, and bread possess a considerable water-binding capacity and are strongly hydrated.

Flour colloids change their water-binding capacity in the process of baking.

In the case of rye flour an increase in the water-binding capacity of the colloids just before transferring into the oven is to be observed whilst in the case of wheat flour doughs no such increase takes place.

A considerable increase of water-binding capacity occurs in the process of bread baking, this effect being more pronounced for wheat than for rye bread.

The scalding of dough sharply increases the water-binding capacity of flour. This increased capacity is retained through all the subsequent stages of the process and during the staling of the bread.

The water-binding capacity of bread colloids is gradually reduced in the process of staling. For rye bread this reduction is more gradual than in the case of wheat bread.

The water-binding capacity of bread and dough colloids depends upon the method of bread making. The same factor strongly influences the modification of water-binding capacity of bread colloids during staling.

A direct relation between the water-binding capacity of dough and hot bread and the loss in baking is to be observed.

Other conditions being equal the loss in drying of bread while in storage is connected with the capacity of bread colloids to bind water.

All the factors which increase the water-binding capacity of colloids (scalding, additions, etc.) decrease the loss in baking, increase the overweight and reduce the loss in drying of the bread during storage.

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REPORT OF THE BREAD JUDGING COMMITTEE

BERT D. INGELS, ROY IRWIN, and QUICK LANDIS

New York Section A. A. C. C., New York, New York

(Read at the Annual Meeting, June 1935)

For more than a year the New York Section A. A. C. C. has been conducting what we have called bread judging contests. The purpose has been to determine whether there was any distinct unanimity of opinion in our group as to what constitutes good flavor (taste and odor) in bread.

Twelve such contests have been held by us. The number of judges at each test has varied from fifteen, when samples were delivered to judges, to fifty at the Toronto Convention. The usual number has been thirty-six.

In ten contests the bread was purchased from grocery stores and was actual commercial bread made by well established bakeries. In one contest the bread was all purchased direct fr n the bakeries and in the other the Fleischmann Company baked the bread under the committee's directions.

Originally we had in mind that we should get all bread direct from the bakery so that it would have equal freshness. When we tried this we actually got warm bread from two of the five bakeries that delivered bread to us. Our results were so pron uncedly favorable to the freshest bread that we discontinued that procedure.

At the start we sampled the whole loaf of bread by using a thoroughly washed *new* tin can of about 500 cc. capacity, cutting off the end crust of the loaf and taking a cylinder of bread free from crust—promptly putting the lid on the can we had what we felt would be a perfect sample of unsliced bread, uncontaminated by pan, crust, or other flavors.

After considerable experimentation we found that the best procedure was to use sliced bread, wrapping two slices together in a $10'' \times 12''$ piece of water-proofed cellophane paper, held together by a rubber band.

According to Dr. Washington Platt, whose advice was very helpful to us, three samples of bread were about the maximum number that could be judged with any degree of accuracy at one time by any judge, although in some tests we used four samples.

In order to eliminate the error in smelling or tasting one bread first and thus rendering the organs of smell and taste less sensitive to the other samples, we staggered the samples so that every possible arrangement was available. In one case we used only two kinds of bread, A and B, and furnished the judges with three samples each, so that the judges had duplicates unknown to them. In this case we had thirty-six judges—a total of 108 samples or 216 slices of bread. The arrangement was as follows:

A	A	B	A	B	A	A	B	B	B	B	A	B	A	B	B	A	A	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	etc.

In this way no two samples had the same number and by sorting out the numbered slips of paper for Sample A and wrapping up those samples including the numbered slip under the cellophane, the chance of error in preparation of samples was minimized—and the same procedure for Sample B.

For three samples the arrangement was:

A	B	C	A	C	B	B	C	A	B	A	C	C	A	B	C	B	A	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	etc.

In both cases it will be noted that it takes six judges to give complete coverage. Therefore, there may be any multiple of six judges. Our usual attendance of around 35 to 40 made the number 36 very convenient.

The judges were told to determine odor first, smelling the lowest numbered sample first and the highest numbered sample last—the same for taste. Instead of grading odor and taste as good, fair, poor, etc., we ranked the grades—1 being best and 3 poorest—and 2 intermediate. Then by making up a sheet of the numbers for each kind of bread and adding these grades, we arrived at a figure that gave us the composite judgment of the whole group.

With three breads, the lowest possible figure for 36 judges would be 36 and the highest possible (and hence poorest bread) figure would be 108. In the case of only two kinds of bread, 54 and 162 would be the extremes.

With each set of three samples for the judges, we furnished score cards made up as follows:

Sample No.	Odor	Taste	Sample No.	Odor	Taste
34			34		
35			35		
36			36		
Sign this portion			Retain this portion		

In all, forty-four lots of bread were judged and over fifteen hundred samples prepared for the judges.

Breads were selected for the following characteristics:

Rich formulas
Medium formulas
Lean formulas
No sugar
Usual sugar
No salt
Usual salt
No shortening
Butter as shortening
Lard as shortening
Hydrogentated cottonseed oil as shortening
40 hour old bread
10 hour old bread.

There were several duplications amongst these tests.

All but three of these contests were held at the section meetings. The bread was judged immediately preceding the evening meal. In two of the contests samples were delivered to the judges' laboratories and in the case at Toronto the bread was judged in the convention hall just before lunch.

A careful study of these twelve contests indicates that we have not made any decided progress in our ability to judge odor and taste in bread.

Methods that have already been worked out respecting smoking, drinking water between samples, etc. must really be rigidly followed. In other words one must become the equivalent of an expert tea taster and a perfumer before he becomes a capable judge of bread flavor.

The New York Section is definitely committed to continue further studies along this line. A special committee consisting of Charles Glabau, Dr. Stroud Jordan, and Dr. H. K. Parker, have undertaken to determine organoleptically the ability of some of us to judge small quantities of various flavoring agents, to the end we may pick out of our group those who are most capable for this judging of bread flavors.

At our October meeting we will again start another series of bread judging contests which we expect to continue monthly through to the May 1936 meeting.

Conclusions

Freshness affects flavor more favorably than any other single characteristic of bread.

The judging of odor and taste in bread is not a simple procedure. Greatest care in preparation and age of samples is necessary. Judges must follow strict rules of conduct.

Only those persons whose senses of smell and taste are keen are capable judges.

VARIETAL AND REGIONAL VARIATION IN DURUM WHEAT STARCHES [1]

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North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Read at the Annual Meeting, June 1935)

Since durum wheats are used principally for macaroni products, the properties of their starches are presumed to be of considerable importance in determining quality. The principal requirement of a good macaroni product, in addition to a creamy yellow color, is good cooking quality. Good cooking quality infers that the product will not break up when cooked but will hold its characteristic shape and will not be sticky or slimy. The protein constituent of macaroni as well as its starch content affects cooking quality, but the role of starch is probably most important.

It is known that wheat starches vary in their properties. Rask and Alsberg (1924) noted differences in the viscosity of pastes made from starch of different wheats. Mangels and Bailey (1933) more recently have shown that starches from different types of wheat show considerable variation when treated with cold gelatinizing agents.

Mangels (1934) studied varietal, regional, and seasonal variations in properties of hard red spring wheat starches. The present paper is a continuation of this study and deals with durum wheat starches.

Experimental

Pure starches were prepared from 12 samples of wheat, representing 5 varieties of durum from the 1933 crop. The wheat was first milled to a 75% patent flour and the starches were prepared from the patent flour. The method of preparation was that described by Mangels and Bailey (1933). The wheats were secured from variety plots at Fargo, the Dickinson Substation, and the Langdon Substation. The varieties selected for study were Kubanka, Mindum, Monad, and Pentad. Pentad was not grown at Langdon, and C. I. 8882, a Mindum-Pentad cross, was substituted. Kubanka and Mindum are

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[2] Contribution from Department of Cereal Technology.

satisfactory commercial amber varieties, while Monad is an amber variety of inferior quality, and Pentad a red durum also of inferior quality.

Ash, Phosphorus, and Nitrogen Content

The ash, phosphorus, and nitrogen contents of the purified starches used are given in Table I.

TABLE I

ASH, PHOSPHORUS, AND NITROGEN CONTENT OF DURUM STARCHES
(Dry Basis)

Laboratory Number	Location	Variety	Ash	P	N
			%	%	%
34–4–18	Fargo	Kubanka	0.206	0.060	0.036
19	"	Mindum	0.204	0.061	0.038
20	"	Monad	0.200	0.059	0.041
21	"	Pentad [1]	0.209	0.057	0.036
22	Dickinson	Kubanka	0.238	0.063	0.037
23	"	Mindum	0.256	0.064	0.041
24	"	Monad	0.209	0.051	0.041
25	"	Pentad [1]	0.206	0.055	0.036
26	Langdon	Kubanka	0.190	0.043	0.030
28	"	Mindum	0.155	0.044	0.030
27	"	Monad	0.170	0.041	0.030
29	"	C.I.8882 [1]	0.236	0.045	0.030
		Regional Average			
		Fargo	0.203	0.060	0.038
		Dickinson	0.234	0.059	0.040
		Langdon	0.172	0.043	0.030
		Varietal Average			
		Kubanka	0.211	0.055	0.034
		Mindum	0.205	0.056	0.036
		Monad	0.193	0.050	0.037

[1] Not included in regional average.

The ash and phosphorus contents of the starches show both regional and varietal variation. The Dickinson grown durum wheat starches contained the most and the Langdon grown durum wheat starches the least ash. The varietal variation at Fargo was not significant, but at Dickinson the Kubanka and Mindum starches showed a significantly higher ash content than the Monad or Pentad starches. Considerable variation in ash content occurred in the starches prepared from the durum wheat grown at Langdon. The order is C. I. 8882, Kubanka, Monad, and Mindum. Apparently there was no consistent varietal variation in ash content, if starches from the three locations are considered.

The phosphorus content was much lower in the starches prepared

from wheat grown at Langdon. Kubanka starch from Langdon contained 0.043% phosphorus as compared with 0.060% and 0.063% phosphorus for starch from this variety when grown at Fargo and Dickinson, respectively. Varietal variation in phosphorus content is more consistent than varietal variation in ash content. Mindum and Kubanka starches showed approximately the same phosphorus content at each of the three locations. Mindum starch was consistently higher in phosphorus content than Kubanka starch, Monad was slightly lower in phosphorus content than Mindum or Kubanka starches prepared from wheat grown at Farago and Langdon but was significantly lower for starches prepared from wheats grown at Dickinson. Pentad starches were lower than Mindum or Kubanka starches in phosphorus content when prepared from wheats grown at both Fargo and Dickinson.

There was no significant varietal variation in nitrogen content. The Langdon grown durum wheat starches were significantly lower in nitrogen content, indicating that these starches were more easily freed from contaminating protein material than the starches made from Dickinson and Fargo grown wheats.

Susceptibility to Malt Diastase

A quantity of air-dry starch equivalent to 5 g. of moisture-free starch was weighed out and placed in a 200 cc. Erlenmeyer flask. A teaspoonful of ignited sea sand was introduced into the flask and an accurately weighed 25 mg. portion of Merck's malt diastase was added, and the flask and contents brought to 30° C. by placing in a thermostat bath. After the flask and contents had reached 30° C., 46 cc. of Sørenson's phosphate buffer (pH 6.0) at 30° C. was added and diastasis allowed to proceed for 1 hour. At the end of one hour the enzyme was inactivated by addition of sulfuric acid and sodium tungstate, bringing total volume of liquid added to 50 cc. Maltose was then determined by the Blish-Sandstedt procedure (1933).

The data for the 12 durum starches are given in Table II and shown graphically in Figure 1.

The susceptibility of the starches from the different durum varieties shows considerable variation for the different locations. Kubanka starches showed relatively less variation than other varieties, ranging from 215.0 mg. for the Fargo grown wheat to 240.0 mg. for the Langdon grown wheat. Mindum starches showed somewhat wider variations, varying from 195.8 mg. to 237.5 mg., while Monad starches varied from 206.5 mg. to 277.5 mg.

The Dickinson grown durum wheat starches showed relatively small varietal variation in susceptibility to malt diastase but Pentad starch

TABLE II

DURUM WHEAT STARCHES

Susceptibility of Durum Starches to Malt Diastase
Maltose found in 1 hr. at 30° C.

Laboratory Number	Location	Variety	Maltose per 5 g. starch
			Mgs.
34–4–18	Fargo	Kubanka	215.0
19	"	Mindum	195.8
20	"	Monad	206.5
21	"	Pentad [1]	270.0
22	Dickinson	Kubanka	239.0
23	"	Mindum	237.5
24	"	Monad	240.8
25	"	Pentad [1]	255.0
26	Langdon	Kubanka	240.0
28	"	Mindum	217.5
27	"	Monad	277.5
29	"	C.I.8882 [1]	285.0

5 g. starch digested with 25 mg. Merck's malt diastase and 46 cc. Sørenson's phosphate buffer (pH 6.0).

Regional Average

Fargo	205.8
Dickinson	239.1
Langdon	245.0

Varietal Average

Kubanka	231.3
Mindum	216.9
Monad	241.6

[1] Not included in regional average.

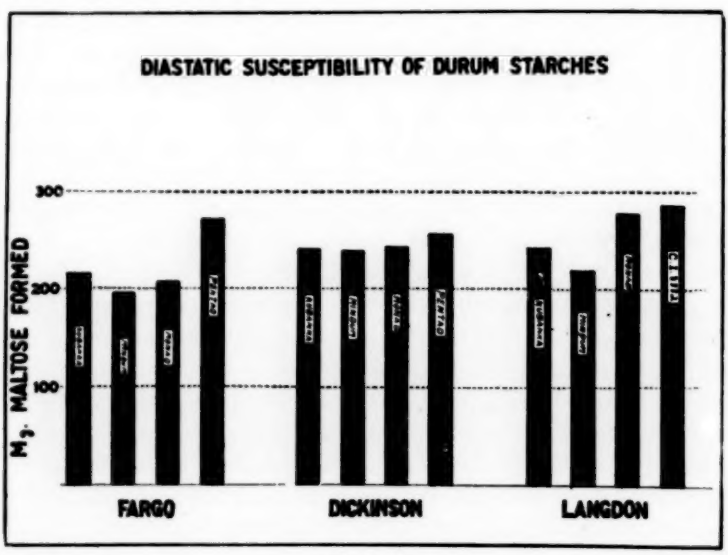

Figure 1.

was more susceptible than the starches from the other three varieties. The Fargo grown durum wheat starches showed relatively small varietal variation except in the case of Pentad starch which was much more susceptible.

Pentad and C. I. 8882 starches were apparently more easily attacked by malt diastase than starches from other varieties, and the Monad starch was more susceptible than Kubanka or Mindum starch, particularly in the case of the starch from the Langdon grown wheat.

Viscosity with Cold Gelatinizing Agents

Three reagents were selected, namely, 0.10 molar NaOH, 1.25 molar KCNS, and 4.5 molar urea, representing respectively a strong base, a neutral salt, and a non-electrolyte. The concentrations used were those found to be suitable from previous studies.

Viscosity was determined by means of a special Ostwald pipette at 30° C., according to the procedure described by Mangels and Bailey (1933). Viscosity is given as ratio of t/tw where t is time for starch preparation and tw is time for distilled water at the same temperature. The starch concentration in all cases was 5%.

Viscosity with 0.10 molar NaOH. The results are given in Table III, and Figure 2.

TABLE III

DURUM WHEAT STARCHES

Viscosity with 0.10 *N* NaOH at 30° C.

Laboratory Number	Location	Variety	Viscosity t/tw				
			0	1 hr.	3 hrs.	6 hrs.	24 hrs.
34–4–18	Fargo	Kubanka	1.1	10.6	21.3	30.5	39.1
19	"	Mindum	1.1	9.3	19.5	27.0	32.0
20	"	Monad	1.1	16.2	28.6	34.0	38.6
21	"	Pentad [1]	1.1	17.7	37.4	52.7	61.2
22	Dickinson	Kubanka	1.0	12.3	18.0	20.4	19.0
23	"	Mindum	1.0	15.8	26.7	31.0	33.8
24	"	Monad	1.1	15.9	29.2	37.1	44.4
25	"	Pentad [1]	1.1	24.8	54.9	71.7	78.1
26	Langdon	Kubanka	1.1	49.0	84.3	100.3	101.8
28	"	Mindum	1.1	9.8	20.3	24.9	26.2
27	"	Monad	1.1	59.0	94.1	106.4	96.8
29	"	C.I.8882 [1]	1.2	10.5	33.9	49.8	59.9
	Regional Average						
		Fargo	1.1	12.0	23.1	30.5	36.6
		Dickinson	1.0	14.7	24.6	29.5	32.4
		Langdon	1.1	39.3	66.2	77.2	74.9
	Varietal Average						
		Kubanka	1.1	24.0	41.2	50.4	53.3
		Mindum	1.1	11.6	22.2	27.6	30.7
		Monad	1.1	30.4	50.6	59.2	59.9

[1] Not included in regional average.

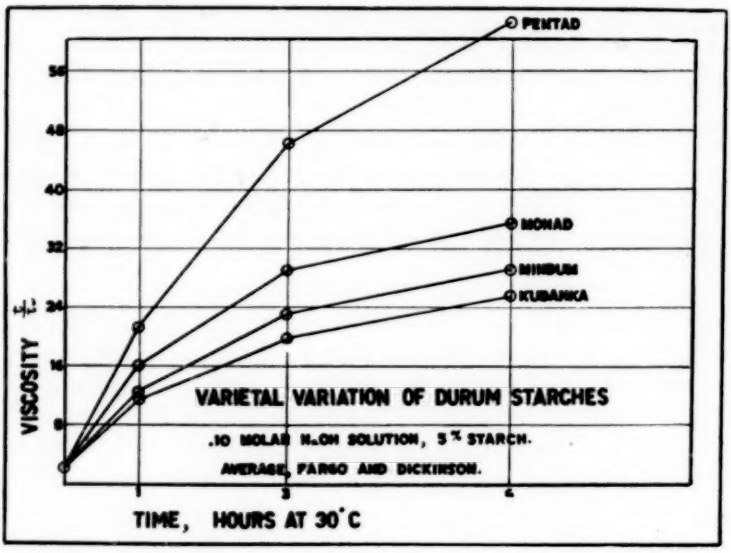

Figure 2.

The regional variation is not large if we exclude the Kubanka and Monad starches made from the Langdon grown wheat. These two starches gave a very viscous preparation not only with 0.1 molar NaOH, but also with the other two reagents. These two starches appeared to be abnormal since the Mindum starch made from Langdon grown wheat showed a viscosity similar to Mindum starch made from wheat grown at Fargo and Dickinson.

Considering the Fargo and Dickinson grown durum wheat starches only (Figure 2), Pentad starch showed a much greater viscosity than the starches made from the other three varieties of durum wheat. Monad starch was consistently higher in viscosity than Mindum or Kubanka. The Fargo Kubanka starch was higher in viscosity than the Mindum starch, but at Dickinson this order was reversed.

From the wheat grown at Langdon, the Monad starch showed a very high viscosity. Kubanka starch was also high in viscosity while the viscosity of the starch from C. I. 8882 was intermediate between that of Kubanka, Monad, and Mindum starches. The Mindum starch from Langdon grown wheat showed a viscosity lower than the Mindum starch made from the Fargo or Dickinson grown wheat.

Viscosity with 1.25 molar KCNS. The results are given in Table IV and Figure 3. The Kubanka starch made from Langdon grown wheat again showed a very high viscosity.

TABLE IV

DURUM WHEAT STARCHES

Viscosity with 1.25 *N* KCNS at 30° C.

Laboratory Number	Location	Variety	Viscosity *t/tw* 0	1 hr.	3 hrs.	6 hrs.	24 hrs.
34–4–18	Fargo	Kubanka	1.2	9.7	16.6	25.5	35.0
19	"	Mindum	1.2	9.5	15.5	18.4	31.9
20	"	Monad	1.3	13.6	22.2	26.6	43.2
21	"	Pentad [1]	1.3	7.7	10.9	12.7	18.4
22	Dickinson	Kubanka	1.2	6.4	10.2	11.9	19.6
23	"	Mindum	1.2	5.5	8.2	10.4	16.4
24	"	Monad	1.3	7.4	12.2	16.3	20.5
25	"	Pentad [1]	1.3	5.8	7.8	10.2	13.8
26	Langdon	Kubanka	1.4	15.9	23.5	34.3	52.7
28	"	Mindum	1.2	7.9	11.8	15.4	23.9
27	"	Monad	1.3	15.6	24.4	32.7	47.9
29	"	C.I.8882 [1]	1.3	8.0	11.7	15.6	23.1
	Regional Average						
		Fargo	1.2	10.9	18.1	23.5	36.7
		Dickinson	1.2	6.4	10.2	12.9	18.8
		Langdon	1.3	13.1	19.9	27.5	41.5
	Varietal Average						
		Kubanka	1.3	10.7	16.8	23.9	35.8
		Mindum	1.2	7.6	11.8	14.7	24.1
		Monad	1.3	12.2	19.6	25.2	37.2

[1] Not included in regional average.

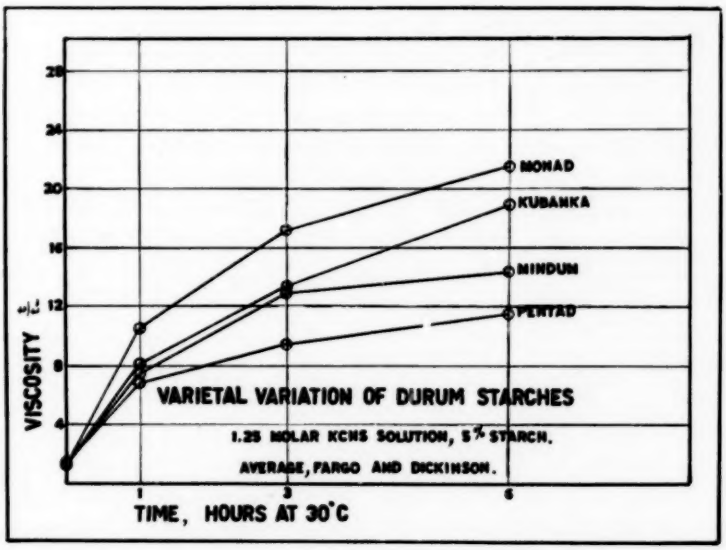

Figure 3.

A rather interesting regional variation was noted in this case. Considering the average of the three varieties, Kubanka, Mindum, and Monad, the durum wheat starches grown at Langdon showed the highest viscosity, and the Dickinson grown durum wheat starches the lowest, with the durum wheat starches grown at Fargo intermediate in value.

Considering the wheats grown at Fargo and Dickinson only, where starches from four varieties are available for comparison (Figure 3), it will be noted that Pentad starch showed the lowest viscosity with 1.25 molar KCNS, while with 0.1 molar NaOH it showed a very high viscosity. Monad starch showed the highest average viscosity. Kubanka and Mindum for the first three hours showed similar viscosities (although Kubanka was consistently higher) but at the six-hour period Kubanka starch from Fargo grown wheat showed a considerable increase in viscosity over Mindum. The order of magnitude for the viscosities of Monad, Kubanka, Mindum, and Pentad starches is entirely consistent for all observations made on the Fargo and Dickinson grown durum wheat starches.

For the starches made from the Langdon grown wheats, the Mindum starch showed a viscosity similar in magnitude to Mindum starch made from the Fargo and Dickinson grown wheat, but the Kubanka starch made from Langdon grown wheat again showed rather abnormal viscosity for this variety.

Viscosity with 4.5 molar urea. The results are shown in Table V and Figure 4.

The regional variation was similar to that shown with 1.25 molar KCNS as gelatinizing agent. Langdon grown durum wheat starches showed the highest viscosity and starches made from Dickinson grown durum wheats were lowest in viscosity, while the Fargo grown durum wheat starches were intermediate in viscosity values. The Langdon Kubanka starch again showed a relatively high viscosity.

Again considering the wheats grown at Fargo and Dickinson only, where starches from four durum varieties were available for comparison (Figure 4), we find Pentad starch showing the lowest average viscosity (similar to the results using 1.25 molar KCNS). The viscosity of the Kubanka and Mindum starches was quite similar, while Monad starch gave a significantly higher viscosity than the other varieties.

The Langdon Kubanka starch, however, was somewhat higher in viscosity than the Monad starch prepared from Langdon grown wheat. The Mindum starch prepared from the Langdon grown wheat showed higher viscosity than Mindum starch from Fargo and Dickinson grown durum wheat, while the C. I. 8882 starch was lower than Mindum in viscosity.

TABLE V

DURUM WHEAT STARCHES

Viscosity with 4.5 molar urea at 30° C.

Laboratory Number	Location	Variety	Viscosity t/tw 0	1 hr.	3 hrs.	6 hrs.	24 hrs.
34–4–18	Fargo	Kubanka	1.8	14.1	19.3	25.0	36.8
19	"	Mindum	1.8	14.7	21.2	26.1	41.5
20	"	Monad	1.8	20.9	29.9	37.8	57.8
21	"	Pentad [1]	1.8	9.2	12.1	13.6	14.3
34–4–22	Dickinson	Kubanka	1.5	8.9	11.5	14.1	20.2
23	"	Mindum	1.6	7.4	9.3	10.7	16.2
24	"	Monad	1.6	9.8	12.8	14.4	18.8
25	"	Pentad [1]	1.8	7.9	9.7	11.2	14.9
34–4–26	Langdon	Kubanka	2.3	26.2	38.4	44.6	65.5
28	"	Mindum	1.5	18.6	22.8	25.2	36.9
27	"	Monad	2.0	26.5	35.9	44.5	62.9
29	"	C.I. 8882 [1]	1.6	11.8	15.4	18.4	23.3
	Regional Average						
		Fargo	1.8	16.6	23.5	29.6	45.4
		Dickinson	1.6	8.7	11.2	13.1	18.4
		Langdon	1.9	23.8	32.4	38.1	55.1
	Varietal Average						
		Kubanka	1.9	16.4	23.1	27.9	40.8
		Mindum	1.6	13.6	17.8	20.7	31.5
		Monad	1.8	19.1	26.2	32.2	46.5

[1] Not included in regional average.

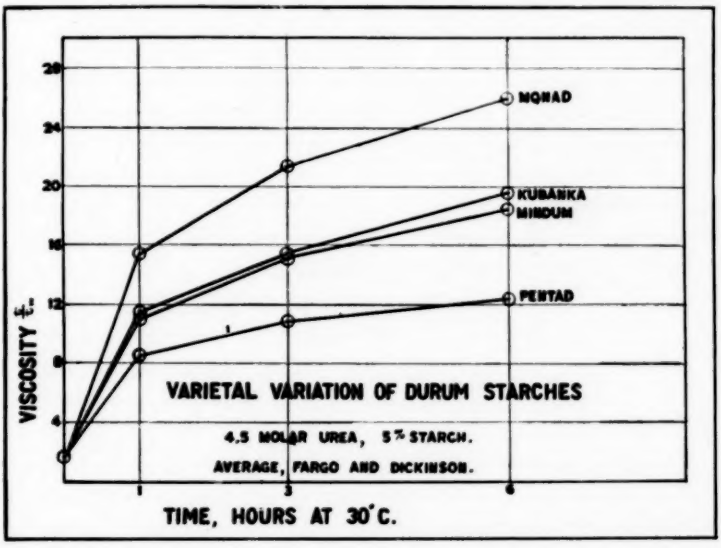

Figure 4.

Viscosity of heat-gelatinized starch preparations. The results are given in Table VI.

TABLE VI

DURUM WHEAT STARCHES

Viscosity of 2% pastes made with boiling water and 0.5 M NaCl solution

Laboratory Number	Location	Variety	Viscosity *t/tw*			
			Distilled H_2O		0.5 M NaCl solution	
			3 hrs.	24 hrs.	3 hrs.	24 hrs.
34–4–18	Fargo	Kubanka	10.8	11.0	5.2	4.0
19	"	Mindum	9.0	9.3	5.3	3.2
20	"	Monad	12.8	13.0	5.6	3.9
21	"	Pentad [1]	5.3	5.5	3.9	3.4
22	Dickinson	Kubanka	10.6	10.2	4.9	4.0
23	"	Mindum	8.9	8.8	4.6	3.4
24	"	Monad	11.0	11.0	4.7	3.9
25	"	Pentad [1]	10.3	10.1	4.2	3.4
26	Langdon	Kubanka	14.8	14.4	5.8	4.6
28	"	Mindum	13.9	13.3	5.8	4.5
27	"	Monad	13.4	12.9	5.3	4.7
29	"	C.I.8882 [1]	8.5	8.3	4.0	3.7
Starch treated with solution at temperature of boiling water 30 minutes. Cooled to 30° C. and observations taken at 3 and 24 hours, after removal from boiling water bath.						
Regional Average						
		Fargo	10.9	11.1	5.4	3.7
		Dickinson	10.2	10.0	4.7	3.8
		Langdon	14.0	13.3	5.6	4.6
Varietal Average						
		Kubanka	12.1	11.9	5.3	4.2
		Mindum	10.6	10.5	5.2	3.7
		Monad	12.4	12.3	5.2	4.2

[1] Not included in regional average.

The starch pastes were prepared by adding boiling water and boiling 0.5 molar NaCl solution to a starch suspension, and placing in an actively boiling water bath for 30 minutes. After 30 minutes the flasks containing the starch pastes were placed in a thermostat bath at 30° C. and viscosity was determined 3 hours and 24 hours after placing in the 30° C. bath. A 2% concentration of starch was used in all cases, since higher concentrations give a paste which is too viscous to be easily handled in the Ostwald pipette.

It will be noted (Table VI) that the viscosity values for starch pastes prepared with boiling water are more than double in most cases when compared with the values for pastes prepared with boiling 0.5 molar NaCl solution (approximately 3% NaCl). The viscosity of

pastes prepared with distilled water showed a relatively small amount of syneresis between 3 and 24 hours, but the pastes prepared with the NaCl solution showed considerable syneresis.

Considering pastes prepared with distilled water, the Langdon starches showed a relatively higher viscosity than the Fargo or Dickinson starches. Of the pastes prepared with the salt solution, however, the Langdon and Fargo grown wheat starches were similar in viscosity when examined 3 hours after removal from the boiling water bath, but since the Langdon grown wheat starches showed less syneresis than the Fargo grown wheat starches the regional relationship for the 24 hour observations were the same as for distilled water pastes.

Of the pastes prepared with distilled water, the Monad starch was highest in viscosity for the Fargo and Dickinson grown wheats. The Kubanka starch prepared from Langdon grown wheat, however, showed the highest viscosity. Mindum starch prepared from wheats grown at all three locations was lower in viscosity than Kubanka starch, and Pentad starch from Fargo grown wheat was very low in viscosity. C. I. 8882 starch prepared from Langdon grown wheat was the lowest in viscosity.

Of the pastes prepared with boiling salt solution, Pentad and C. I. 8882 starches showed the lowest viscosity. For starches prepared from wheats grown at Fargo and Dickinson, Mindum starch showed greater syneresis than Kubanka starch. The use of salt solution decreased the varietal variation, but indicated that Pentad and C. I. 8882 starches were different from the others.

Discussion

The properties of durum wheat starches are evidently subject to both varietal and regional variation. The ash and phosphorus contents showed variation, and there was considerable variation in the susceptibility to malt diastase. The viscosity of cold gelatinized and heat-gelatinized preparations showed considerable variation.

There is some indication that low phosphorus content is associated with high susceptibility to diastase, but Kubanka and Mindum starches prepared from wheat grown at Dickinson were higher in both phosphorus content and diastatic susceptibility than starches prepared from the durum wheats grown at Fargo.

Correlation by ranks gave a coefficient of approximately + .70 between diastatic susceptibility and viscosity with NaOH (6 hour observation), but diastatic susceptibility and viscosity with KCNS or urea solutions or by heat gave negative coefficients.

Correlation by rank of viscosity with NaOH with viscosity with KCNS, urea and hot water gave values of + .30; + 0.18; and + 0.09

respectively. Correlation of viscosity with KCNS and viscosity with urea and hot water, however, gave values of + .92 and + .61 respectively.

Viscosity with NaOH is, therefore, to some extent related to diastatic susceptibility of the starches, while viscosity with such cold gelatinizing agents as KCNS and urea is related to properties of paste prepared with boiling water.

Durum starches in general are more susceptible to diastase and give higher viscosity values than hard red spring wheat starches. The varieties represented in the starches examined consisted of two very satisfactory commercial varieties, Kubanka and Mindum; two unsatisfactory commercial varieties, Monad and Pentad; and a new variety whose characteristics are not well known, C. I. 8882.

Pentad and C. I. 8882 starches are evidently more susceptible to diastase than starches of other varieties. Pentad starch showed a very high viscosity when treated with 0.1 molar NaOH and with exception of the wheat grown at Langdon, Monad starch showed a higher viscosity than Kubanka or Mindum starch. Since, in general, Kubanka and Mindum macaroni have better cooking qualities than Pentad or Monad macaroni, it may be that an extremely high viscosity is undesirable.

It is interesting to note that while Pentad starch showed high viscosity with NaOH, it is among the lowest in viscosity when treated with KCNS solution, urea, boiling water or boiling salt solution. Pentad starch showed consistently low viscosity when treated with boiling 3% salt solution and the same is true for C. I. 8882 starch. Since macaroni products are generally cooked with salt this difference may have some significance.

The present study would indicate that durum starches are probably subject to greater regional variation due to seasonal climatic differences than hard red spring wheat starches (Mangels, 1934). The 12 starches studied were prepared from wheat of the 1933 crop, and the growing season at all three locations was abnormally dry and hot. Dickinson actually had more rain during the growing season than Fargo, and Langdon less than Fargo. The Langdon wheats were seeded later and matured later than the Fargo or Dickinson wheats, and this may account for some of the peculiarities of the Langdon starches.

Summary

The properties of durum wheat starches show both regional and varietal variation.

There is some indication that low phosphorus content may be associated with high susceptibility to diastase, but the data are not consistent.

There is a relationship between viscosity with NaOH solution and susceptibility to diastase, but viscosity with KCNS or urea solutions or with heat gelatinized preparations evidently has no relation to diastatic susceptibility.

Viscosity with KCNS solution compared with viscosity of urea and heat gelatinized preparations shows significant correlation, but viscosity with NaOH solution is not significantly correlated with viscosity of other preparations.

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MEASUREMENT OF RESPIRATION IN FLOUR[1]

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(Read at the Annual Meeting, June, 1935)

The respiration of cereal grains is familiar as a naturally occurring function of all living matter. There have been many studies of the respiration of wheat and it has been found that at the higher moisture percentages, even just above 15 percent, it takes only a few days for mold respiration to overshadow entirely the respiration of the wheat itself.

In dealing with respiration of flour, the question first arises, Is flour living and does it have any true respiration? Apparently there is enough of the enzymes responsible for respiration left in the flour after milling to give true respiration at a rather low rate.

Only one reference to previous work on flour respiration was found and most of that work deals with higher moisture contents, at which that of bacteria and molds probably is the chief or almost the entire respiration present. The study of flour respiration was taken up because of its interest as a possible explanation for the phenomenon which has been described as the " sweat " in flour. If there is such a change in flour, respiration is the only simple explanation for it.

Respiration in flour is a very slow production of carbon dioxide. Ordinary methods of measuring respiration in grains, or in most plant materials, are entirely inadequate for measuring respiration in flour. For that reason, it was necessary to arrange a special apparatus, the main features of which were suggested by the method of Spoehr[2] for measuring the respiration of single leaves. By this method the carbon dioxide produced is absorbed in a small amount of barium hydroxide and the strength determined by the change in conductivity of the barium hydroxide. This gives an accuracy far higher than can be obtained either by weighing absorbed carbon dioxide or by volumetric measurement of the carbon dioxide. The apparatus developed is shown as Figure 1.

In dealing with these very small quantities, it is first necessary to absorb completely the carbon dioxide from the air that is passed through

[1] Contribution No. 51, Department of Milling Industry.

[2] Spoehr, H. A., and J. M. McGee, Studies in plant respiration and photosynthesis, Carnegie Institution of Washington Publication No. 325.

the flour to carry out carbon dioxide produced in the flour. The air is first washed through hydroxide solution. After emerging from the storage container, the air passes through a tube of Ascarite and then

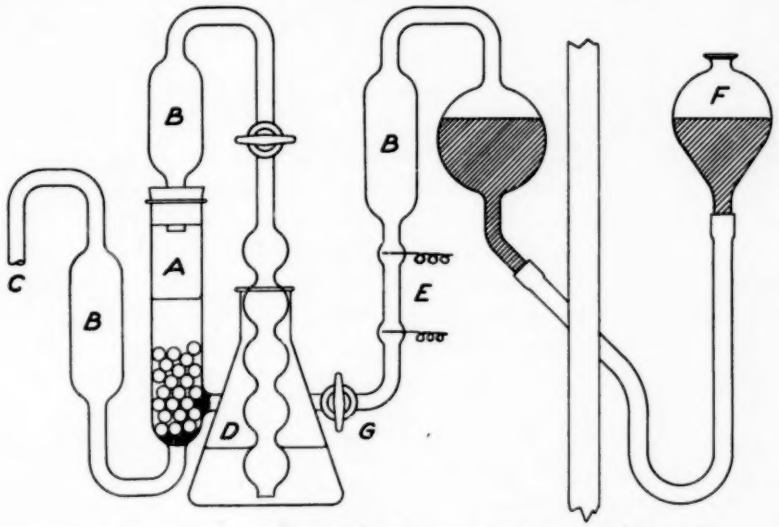

Fig. 1. Absorption tower and conductivity cell for determination of carbon dioxide.

through two more washes with sodium hydroxide; followed by a solution of sulfuric acid adjusted to have a vapor pressure slightly above the vapor pressure of the flour being studied. Passage of the air is by pressure at the beginning of the system rather than by suction at the end, because most of the resistance comes before the air passes through the flour. After this purification the air goes to the top of a calcium chloride tower holding 250 g. of flour. The dead space in the bottom of this tower is filled with paraffin to avoid a hang-up of carbon dioxide there.

After passing through the column of flour, the air is passed through a one-tenth normal sulfuric acid solution to add vapor to the air so that it will not concentrate the barium hydroxide solution by evaporation instead of diluting it by precipitating out barium carbonate. From this dilute sulfuric acid solution, the gas enters into the apparatus at *C* and bubbles through the barium hydroxide solution in tower *A*, which is designed to operate with either 10 or 15 cc. of solution. The packing of glass beads insures complete absorption of carbon dioxide. The beads are kept out of the small tubes by bits of glass wool, which should be placed in position very loosely to avoid clogging with barium carbonate. The gas exit is under a solution of sodium hydroxide in flask *D*, which may be protected from the carbon dioxide of the air by a layer

of white kerosene or light mineral oil. The use of a bulb tube entering this insures scrubbing with sodium hydroxide if any air should be accidentally drawn back into *A*.

Safety bulbs, *B*, *B*, *B*, insure against accidental loss of the standard barium hydroxide. The strength of this solution, and thus the amount of carbon dioxide absorbed, can be determined at any convenient interval by drawing part of the solution into the conductivity cell *E*, mixing it first by two or three passages back and forth. This manipulation is facilitated by placing the mercury leveling bulb *F* and an extension handle to the stopcock *G* outside the constant temperature chamber, while a window allows the process to be watched.

The constant temperature chamber is an air bath with a fan for continuous circulation, heated by electric elements and cooled by the radiator from an automobile heater through which cold water can be circulated. A specially constructed bimetallic thermostat holds the temperature within 0.05° C. as measured by a mercury thermometer. This accuracy seems sufficient for the present purpose, but placing a water jacket around the conductivity cell would assure greater accuracy.

Only a few results obtained by this method are at present ready to report. The daily production of carbon dioxide per 100 g. of flour at 25° C. was approximately 2/1000ths mg.; and at 35° C., approximately 6/1000ths mg. Expressed in cubic centimeters, that corresponds to about 1/1000th cc. and 3/1000ths cc., respectively, of carbon dioxide produced by 100 g. of flour in one day, a quantity too small for convenient volumetric measurement.

There was some indication of an abnormally high respiration that might be due to some process such as sweating in flour, but at present the data are entirely too meager to draw any such conclusion. Many things might happen in measuring such a small quantity, consequently a great deal of repetition is necessary. If a few insects should happen to get into one of the towers they would give off more carbon dioxide than several weeks of respiration of the flour itself.

BOOK REVIEWS

Organic Chemistry. By Howard J. Lucas, Associate Professor of Organic Chemistry, California Institute of Technology. 686 pages. American Book Company, New York, N. Y. Price $3.60.

This new text-book, designed for a full year's course in organic chemistry, presents the subject matter in a systematic and logical manner. Emphasis has been placed on the reactions of classes of compounds rather than upon those of individual compounds, and wherever possible relationships to inorganic compounds have been indicated.

One of the distinctive features is the discussion, in the opening chapters, of atomic and molecular structure and the theory of valency. Those newer concepts of matter that have a direct bearing on the structure and reactions of organic compounds are given. The topics include the electron arrangements of the first eighteen elements of the periodic system, bond energies and the various types of bonds, and the principles of resonance and of minimum kernel repulsions applied to the determination of molecular structure. The properties of polar, nonpolar, and dipolar substances are also discussed. These physical principles are not neglected in the later pages of the text, but are extensively used wherever applicable.

The arrangement of topics is fairly conventional. Perhaps the greatest departure from the usual text-book is the deferred treatment of some compounds of mixed functions, such as hydroxy, amino, and keto acids. These are taken up after the simpler aliphatic and aromatic compounds and are followed by such specialized topics as proteins, carbohydrates, terpenes, heterocyclic compounds, alkaloids and drugs, and dyes.

Equations are used freely throughout the work, and full graphic formulas are given wherever necessary—a decided advantage, as so many text-books unwisely economize in this matter. Another pleasing and helpful feature is the abundance of problems; those in which structural formulas are to be deduced from given experimental data are especially to be commended.

There are excellent charts dealing with type reactions of the most important classes of compounds and a good many tables of physical properties. Noteworthy also are the tables dealing with orientation in the benzene ring and that showing stereoisomeric possibilities.

While the text is quite thorough and up-to-date in its treatment of reaction mechanisms, there are several subjects the reviewer would like to have seen mentioned. With so many publications appearing on vitamin D, the sex hormones, and other closely related compounds, a brief discussion of cholesterol and its relation to important biological products might well have been included. The chlorination of pentane is mentioned, but the commercial production of pentanols from the chloropentanes is omitted. The cellosolves are described, but the closely related carbitols have not been mentioned. Since ethylene and acetylene are the starting materials for many synthetic compounds available commercially today, charts showing the derivation of these compounds from the gases might have been useful. More extensive cross references would also have been of considerable help to the student. For example, the catalytic oxidation of benzene to yield maleic anhydride is given, but in the paragraph on maleic acid nothing is said of its formation.

These are minor criticisms, however. The text probably represents the best application of the newer physical chemistry to organic chemistry which has so far appeared in a book intended primarily for undergraduates, and brings together much material which could otherwise be found only by reading a good many recent original papers.

Throughout the text the late Professor Crafts' name is given as Craft.

H. L. HALLER

Die Bestimmungen der Wasserstoffionen-Konzentration (pH) und deren Bedeutung fur Technik und Landwirtschaft. By Holger Jörgensen. Published by Theodor Steinkopf, Dresden and Leipzig, 1935. 264 pp.

This manual is divided into three major parts which cover (I) the physicochemical theory involved in hydrogen-ion phenomena, (II) electrometric and colorimetric methods of measurement, (III) technical aspects, which cover (*a*) the direct and (*b*) the indirect action of the hydrogen-ion, and (*c*) mechanisms in which the influence of pH is evident but less understood.

The first two parts are in a form that renders them singularly useful to the serious student of this subject. The presentation is thoroughly modern and logical. Mathematical treatments are arranged clearly and in orderly sequence. Various mechanical devices are described, including the hydrogen, quinhydrone, antimony and glass electrodes.

Practical applications of pH control in various industrial processes and laboratory operations occupies the last third of the volume. Included in this section are pH effects upon enzyme activity as with invertase; diastase in bread production; textiles; leather manufacture; yeast and alcoholic fermentation; microorganisms, including rope-producing in bread; color of various materials such as paper, leather, and beer, and various other pH controls.

These latter discussions are brief, perforce, as imposed by limitations in the size of the manual. They appear to be very useful to one who wishes to survey these several fields insofar as hydrogen-ion concentration effects are concerned.

C. H. Bailey

ERRATA

THE MECHANISM OF DOUGH FERMENTATION: NOTE ON A METHOD FOR COUNTING YEAST CELLS IN A FERMENTING DOUGH, by A. G. Simpson. Volume XIII, page 53. Experiment 6 (*b*) should read:

(*b*)	77, 90, 86, 92, 107 106, 113, 110, 105, 114	average of 100, or 0.69 cell per standard volume

and for Experiment 7, the last half should read:

	Yeast cells per standard volume	
	Dough	*Control*
Flour No. 1	1.00	1.16
Flour No. 2	0.97	1.05
Flour No. 3	1.30	1.30

The number of yeast cells in one gram of yeast, calculated from the data given in Experiments 1 to 7 inclusive, is on the average 1.72×10^{10}, ranging from 1.38×10^{10} to 2.02×10^{10}.

From the figures given in Turley's paper, the calculated number of cells per gram of yeast would be 7.87×10^{9}.